A STUDY OF THE BIOLUMINESCENCE OF A DEEP SCATTERING LAYER ORGANISM (EUPHAUSIA PACIFICA) IN MONTEREY BAY, CALIFORNIA

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THESIS

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March 1974

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by

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Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE IN OCEANOGRAPHY

from the

NAVAL POSTGRADUATE SCHOOL
March 1974

ABSTRACT

A vertical migration tube (VMT) was designed and constructed as an instrument to be used with a photomultiplier light detector to make in situ mesopelagic studies of deep scattering layer vertical migration organisms. Initial tests of the unit demonstrated the feasibility of its use in the marine environment. Three major problems of marine bioluminescent studies using an underwater photomultiplier detector are resolved in part by the use of the VMT with this sensor. Mesopelagic euphausiid crustaceans captured in the upper 100 meters of the water column at night decreased their bioluminescent flash rates when lowered in the water column and exposed primarily to pressure and temperature changes. There may be an increase in euphausiid bioluminescent flash rates when stimulated by other bioluminescent organisms. Laboratory test equipment and laboratory methods were developed to permit quantitative measurements of euphausiid bioluminescent output. Laboratory tests of Euphausia pacifica indicated a greater bioluminescent response to a standard flash stimulus during midnight tests as opposed to noon tests. Laboratory tests of bioluminescent activity during periods of moulting indicated greater than average response to a photoflash stimulus just prior to moulting and less than average response just after moulting.

TABLE OF CONTENTS

I. INTRODUCTION ---------- 9

II. NATURE OF THE PROBLEM AND OBJECTIVES ---------- 13

A. NATURE OF THE PROBLEM ---------- 13

1. Equipment ---------- 13

2. Methods ---------- 14

B. OBJECTIVES ---------- 15

III. INSTRUMENTATION ---------- 19

A. ELECTRONICS ---------- 19

B. VERTICAL MIGRATION TUBE ---------- 24

C. LABORATORY TEST EQUIPMENT ---------- 25

IV. PROCEDURES, TESTS AND RESULTS ---------- 31

A. AT SEA ---------- 31

1. General Procedures ---------- 31

2. At-Sea Tests and Results ---------- 32

a. Equipment Checkout ---------- 32

b. Ambient Light Measurements --------- 33

c. VMT with Euphausiids (Excluding Ambient Light) ---------- 37

d. VMT with Euphausiids (Including Ambient Light) ---------- 41

B. LABORATORY ---------- 41

1. General Procedures ---------- 41

2. Laboratory Tests and Results ---------- 45

a. Spontaneous Activity Test ---------- 45

b. Stimulated Bioluminescent Activity Versus Ambient Light ---------- 47

c. Stimulated Bioluminescent Activity as a Function of Temperature, Moulting, and Time of Day ---------- 50

d. Sympathetic Bioluminescent Response ---------- 90

V. DISCUSSION ---------- 92

A. AT-SEA RESULTS ---------- 92

B. LABORATORY RESULTS ---------- 97

C. GENERAL DISCUSSION AND FUTURE USE OF VMT ---------- 100

VI. CONCLUSION ---------- 103

APPENDIX A Chart of ACANIA Cruises ---------- 121

APPENDIX B Chart of Euphausiid Species Found at CalCOFI Station 3 ---------- 122

APPENDIX C XBT at CalCOFI Station 3 ---------- 123

APPENDIX D Spectral-sensitivity Characteristic of 1P21 (S-4 response) for a radiant flux from a tungsten source at 2870°K.-------- 124

APPENDIX E Calibration Curves for 1P21 Tube -------- 125

LIST OF REFERENCES ---------- 126

INITIAL DISTRIBUTION LIST ---------- 129

FORM DD 1473 ---------- 131

LIST OF FIGURES

1. Map of Monterey Bay showing CalCOFI 1 Station, CalCOFI 3 Station, Hopkins Marine Station (HMS) and Naval Postgraduate School (NPS). (Depths in fathoms) ---------- 18

2. Spectral sensitivity characteristics and typical sensitivity and current amplification characteristics of 1P21 phototube. (From RCA Handbook of Photomultiplier Tubes) ---------- 20

3. Photomultiplier tube in underwater housing ---------- 22

4. Circuit for photomultiplier light detector showing subsurface (A) and surface (B) units ---------- 23

5. Vertical Migration Tube with and without photomultiplier sensor attached ---------- 26

6. Euphausiid showing general terminology (After Mauchline and Fisher, 1969) ---------- 27

7. Laboratory test apparatus showing sequence followed in testing euphausiids. A. Euphausiid in tube B. Euphausiid in test chamber C. Test chamber with euphausiid in reflector D. Apparatus in position E. Spontaneous test ---------- 29

8. Chart recording of dark current of photo-multiplier light detector at 100 meters depth ---------- 34

9. Bioluminescence of environment at 25 meters (A), 50 meters (B), 75 meters (C), and 100 meters (D) ---- 35

10. Bioluminescence of environment at 125 meters (A), 150 meters (B), 200 meters (C), and 250 meters (D) -- 36

11. Bioluminescence of 10 euphausiids at 25 meters (A), 50 meters (B), 100 meters (C) and 150 meters (D) and 50 meters (E) and 25 meters (F) on return trip to surface ---------- 39

12. Continuous trace of bioluminescence of environment from 50 meters (top right) to 300 meters (lower right) and return to 50 meters (lower left). Read right to left. 8:00 PM ---------- 40

13. Continuous trace of 10 euphausiids from 50 meters (right side) to 150 meters (left side). Not exposed to outside light. 9:00 PM ---------- 42

14. Continuous trace of 10 euphausiids from 50 meters to 125 meters (right to left). Exposed to outside light. 9:30 PM ---------- 43

15. Spontaneous bioluminescence test results --------- 46

16. Example of spontaneous activity of 15 euphausiids ---------- 48

17. Example of complete record (A) of stimulated euphausiid and example of almost (actually active for 2 more minutes) complete record (B) of stimulated euphausiid ---------- 52

18. First four tests of laboratory euphausiid #5. No. is day of month, n = noon test, m = midnight test ---------- 54

19. Second four tests of laboratory euphausiid #5. No. is day of month, n = noon test, m = midnight test ---------- 55

20-40. Daily activity charts for euphausiids. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes ---------- 57-77

41-48. Individual euphausiid averages for parameters indicated (PR MLT implies "prior to moulting", AFT MLT implies "after moulting") ---------- 79-86

49-50. Group averages by temperature group for parameters indicated (PR MLT implies "prior to moulting", AFT MLT implies "after moulting") ----- 87-88

51. Chart of bioluminescent activity versus depth of environment and test groups of euphausiids for ACANIA cruises 74-14, 74-19 ---------- 95

52. Bioluminescent records of environment and individual E. pacifica. a. Environment at 100 m depth; b. 10 euphausiids at 50 m in VMT; c,d. large euphausiid flashes at 100 m in VMT; e,f. laboratory recordings of light output of individual E. pacifica ---------- 96

LIST OF TABLES

1. Record of the measured parameters of the daily bioluminescent activity of *E. pacifica.* ------ 106

2. Averages of each measured parameter of the daily bioluminescence for each *E. pacifica.* ---------- 116

3. Averages of each measured parameter for each temperature group of *E. pacifica.* ---------- 120

ACKNOWLEDGEMENTS

This study required work in several realms of science, many of which required specialized knowledge and facility which I did not possess. I wish to express special thanks to my two advisers; LCDR Calvin R. Dunlap for his help with the ecology of deep scattering layer organisms and many suggestions in laboratory and field work, to Professor Stevens P. Tucker for help with the electronics and suggestions in the field of light properties, and to both of them for numerous suggestions in the preparation of this thesis. Dr. George Evans offered suggestions and asked thoughtful questions concerning the endogenous rhythms of organisms.

The captain, W. W. Reynolds, and crew of the ACANIA are among the finest group of men that I have had the privilege to work with. In eighteen cruises with them I received complete cooperation and help at all times.

I also wish to thank Stanford University for the use of Hopkins Marine Station and Dave Brascher of that facility for help on numerous occasions. The NPGS Oceanography Department's Dana Mayberry and the NPGS machine shop fabricated parts on many occasions.

Finally, to my daughter, April, who spent many hours in a cold laboratory monitoring recordings of bioluminescent activity I express my gratitude.

I. INTRODUCTION

Bioluminescence is the emission of light from living organisms as the result of internal oxidative changes. It is a characteristic of many pelagic marine organisms, yet it is one of their most poorly understood features (Tett and Kelly, 1973). Long recognized as a phenomenon in the surface waters of the ocean or in deep sea benthic fish (Beebe, 1934), the great extent of bioluminescent activity was not fully realized until the development of the photomultiplier light detector. This sensor, which can measure light intensities to below 10^{-7} W/cm^2, has been used by several investigators (Clarke and Wertheim, 1956; Clarke and Backus, 1956; Clarke and Hubbard, 1959; Kampa and Boden, 1954; Breslau and Edgerton, 1958; Clarke and Kelly, 1965; Neshyba, 1967; Rudykov, 1968) in ambient light and bioluminescent studies. These studies reveal the omnipresence of bioluminescence in the oceans and suggest that it must have considerable ecological and behavioral significance.

Light production is known to occur with a degree of certainty in ten phyla and about 35 orders of marine animals (Nicol, 1967). At least seven phyla contribute luminescence to the photic environment of the marine planktonic community (Boden and Kampa, 1964). The greatest development of luminescent organs in animals and the greatest proportion of luminescent forms are found in the mesopelagic regions of the oceans (Tett and Kelly, 1973). Many of the primary

organisms thought to compose the acoustic "deep scattering layer" (DSL) are capable of bioluminescence.

The DSLs are a regular biological and acoustic feature of all the world's oceans. They ascend at sunset to near the sea surface and descend at sunrise to depths between 200 and 1000 meters. During this cycle they appear to follow selected isophots (Kampa and Boden, 1954). The organisms composing the DSL are among the most numerous on earth and comprise part of a great and complex pelagic ecology. They are basic to the oceanic food chain and are a primary source of volume reverberation of sound energy in the ocean. The acoustic volume scattering strength within the layers can be -70 to -80 db (at 24 kHz) but is variable within the layer. At lower frequencies the volume scattering strength is variable and unpredictable (Urick, 1967). These properties make the DSL an important consideration in fathometer trace interpretation and in military subsurface acoustic operations.

The photomultiplier light detector was used by Clarke and co-workers (Clarke and Wertheim, 1956; Clarke and Hubbard, 1959; Clarke and Breslau, 1960) and by Kampa and Boden (1954) to measure the downwelling irradiance of sunlight at great depths. These investigators noticed the sensor also detected bioluminescence and proceeded to investigate its distribution. Further work by these and other investigators (Clarke and Backus, 1956; Boden and Kampa, 1957; Clarke and Kelly, 1965) has greatly increased our knowledge of the vertical distribution of luminescent pelagic animals. Interrelations

between vertical migration of the organisms, the rate and magnitude of luminescent flashing, and diurnal changes in the intensity with depth of the downwelling sun- and sky-light have been documented, but the organisms responsible have not been identified directly.

A midwater trawl used at sensor depths catches bioluminescence organisms, but the identity of a species that luminesces within range of the light sensor cannot be known. Breslau and Edgerton (1958) and Breslau, Clarke and Edgerton (1967) attempted to photograph the luminescent organisms. A photomultiplier detector was used which, upon being stimulated by the flash of a luminescent animal, triggered an electronic flash and camera combination. A few photographs were obtained of some coelenterates and crustaceans, but most of the photographs showed no recognizable organisms.

The major objective of this study was to develop and use an in situ system for studying the bioluminescence of a given organism at depth. Work by Barham (1956) in Monterey Bay showed that Euphausia pacifica, a luminescent euphausiid, was a major organism of the bay's DSL. This crustacean is very probably the most common and widespread of the euphausiid species in the North Pacific (Barham, 1956) and it plays a key role as a major link between the products of photosynthesis and higher members of the food chain. E. pacifica can be maintained in a laboratory for several weeks with minimum maintenance, and, therefore, prolonged laboratory tests are possible (Lasker and Theilacker, 1965).

Investigators have generally neglected in situ testing of pelagic marine organisms. Hardy and Paton (1947) conducted a series of in situ vertical migration tests on planktonic animals using long glass cylinders at different levels in the sea. The cylinders had trap doors separating a number of internal compartments. Messenger weights were used to control the doors and allow animals to move up or down within the cylinder in a given period of time under different conditions of ambient light and depth. The cylinder was sealed so that there was no exchange of water with the outside environment. Reviews of marine bioluminescence by Boden and Kampa (1964) and Tett and Kelly (1973) do not mention in situ studies.

II. NATURE OF THE PROBLEM AND OBJECTIVES

A. NATURE OF THE PROBLEM

1. Equipment

Marine bioluminescence falls in the visual light range, typically peaking between 470nm and 500nm with total (all frequencies) power density at one meter distance ranging from 10^{-11} to 2×10^{-7} $\mu W/cm^2$ (Nicol, 1962). At such low light levels the small input light signal is difficult to amplify with a good signal-to-noise ratio except with a photomultiplier tube.

A photomultiplier produces an electric current which is directly proportional to the amount of light falling upon the sensor and quasi-logarithmically proportional to the high voltage across the tube. The spectral sensitivity depends mainly upon the composition of the photoreceptive surface, and the amplification depends upon the supply voltage and the geometry of the internal diodes. Voltage control circuitry (to lower the voltage across the electrodes as the current increases) or a system of stops and filters (to control the light entering) must be used to achieve a large dynamic light range.

The use of a sensitive, high voltage instrument in the marine environment poses some design problems. Sensors designed for in situ use are a compromise between sensitivity, available components, and the need for rugged construction (Tett and Kelly, 1973).

The photomultiplier light detector and associated components must be protected by a pressure-resistant housing and normally connect with the ship via a long electric cable. A photomultiplier tube operates from a high voltage DC power supply, typically 1000 VDC or higher. This must be sent down the cable to the sensor from a surface supply or carried in situ in the form of a battery pack or local high voltage supply. The instruments present difficulties in maintenance because of high input voltages, low current sensitivity, and high photomultiplier output impedance, but these can be overcome by proper design.

2. Methods

Once a light detector is in the ocean the experimental techniques employed become of paramount importance. The sensor is suspended on a cable and, except in dead calm conditions, experiences a vertical oscillation which provides an artificial stimulus to the organisms and causes a bioluminescent response (Mauchline and Fisher, 1969). The distance and the angle from the organism to the sensor are unknown, and, therefore, the absolute light output of the organisms cannot be determined. The greatest failing has been the inability of the investigator to identify the organism producing the light. Attempts to solve these problems have not met with success, and they remain largely unresolved (Mauchline and Fisher, 1969; Tett and Kelly, 1973).

B. OBJECTIVES

The primary goal of this investigation was to attempt to circumvent these problems with new methods and equipment and, possibly, to extend the present limited knowledge of marine bioluminescence. A second objective was to attempt to relate the results (or possible future results) to military operations.

The great prevalence of bioluminescent organisms in pelagic communities (Nicol, 1967) and the increase of bioluminescent activity recorded within the DSL (Boden and Kampa, 1957) indicate that bioluminescence may be an important parameter that could be used to locate the DSL. The use of a passive light detector versus an active sonar to locate an acoustic scattering layer would give a submarine a great advantage in trying to remain undetected.

Brown (1970) examined the possibility of detecting submerged submarines from the bioluminescence induced by their passage through bioluminescent-active waters. The depth at which a submarine may be detected in this manner from air depends upon three environmental factors: the luminance of the water background, the luminance of the bioluminescence excited by the submarine surface, and the attenuation of the bioluminescent light by the water in the path between the submarine and the observer (Brown, 1970). The most uncertain parameter is the brightness of the induced bioluminescence. Brown (1970) predicted detection to 120 meters depth with the aid of a light amplification device

in clear ocean water on a dark night. This study indicates that more in situ research is needed.

The present study was conducted because it was felt that extrapolation of the results of laboratory testing of animals of the deep ocean is of limited validity, i.e. many of the results obtained in the laboratory are suspect due to the artificial environment. Once an animal is removed from its natural environment and placed in an artificial one there is a 'zoo-effect'. This is the change in the animal's responses and behavior effected by its laboratory environment. The environmental changes may be obvious changes, such as the physical changes in the ocean which DSL organisms are subjected to in diurnal vertical migration and which are difficult to reproduce in a laboratory. They may also be subtle and involve an environmental mechanism which triggers a circadian rhythm. Since in most cases the natural responses of the animal are not known (usually that is what we are trying to determine) the extent of the 'zoo-effect' is unknown. An in situ approach is therefore necessary for many experimental studies, and concurrent in situ and laboratory studies may be even more valuable.

The following specific objectives were set:

1. Capture, identify and maintain Euphausia pacifica in a laboratory for experimental testing;

2. Design and build:

    a. An apparatus having a photomultiplier detector to measure and record bioluminescence;

b. Laboratory and in situ test equipment to obtain quantitative measures of light output by individual E. pacifica;

c. Make at-sea in situ tests of DSL organisms (specifically E. pacifica) from R/V ACANIA (the Naval Postgraduate School's research vessel) in Monterey Bay (Figure 1, Station 3); and

d. Supplement the at-sea tests with laboratory tests of bioluminescence from E. pacifica against such parameters as temperature, time, moulting frequency, oxygen, pH, and light.

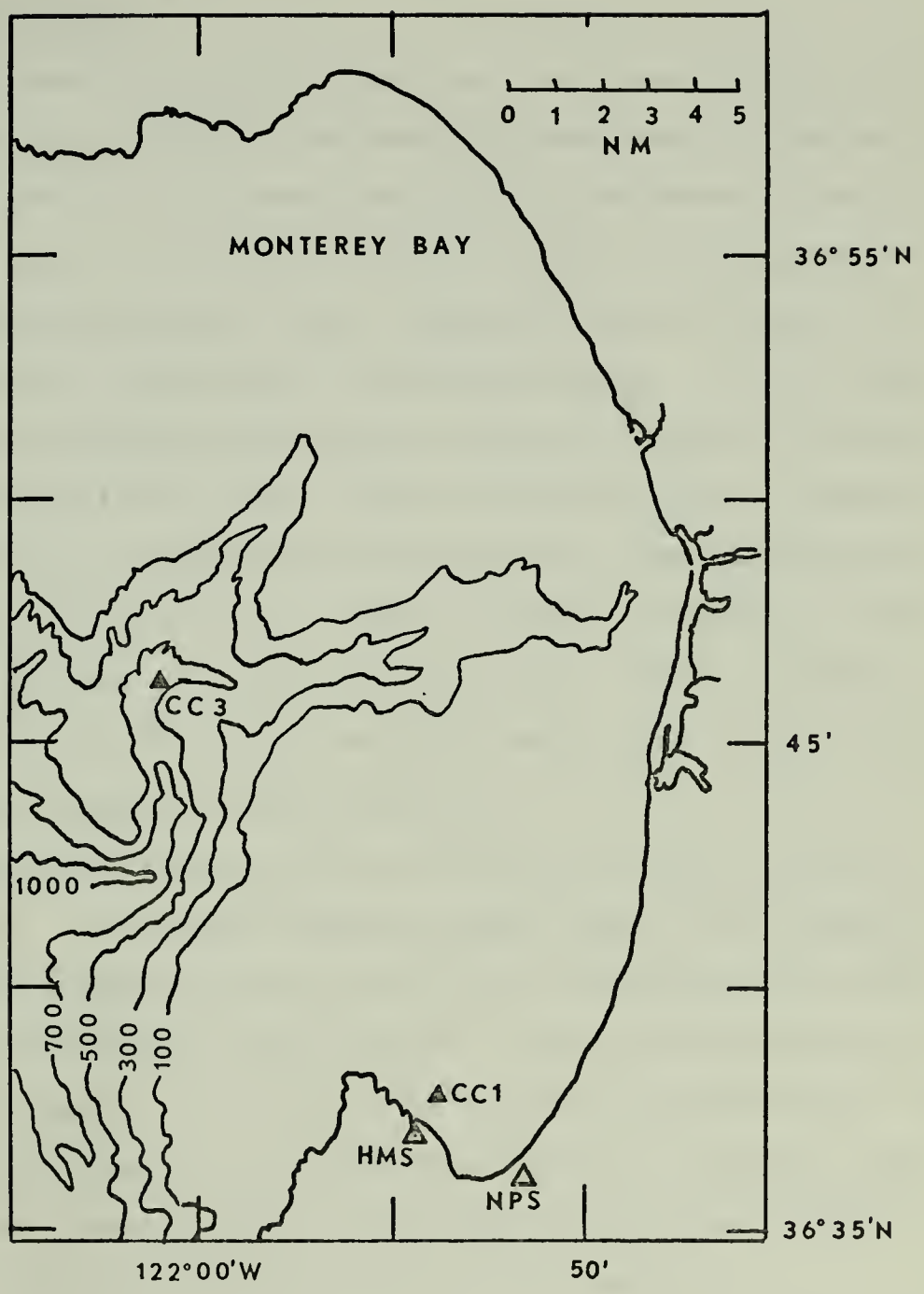

Figure 1. Map of Monterey Bay showing CalCOFI Station 1, CalCOFI Station 3, Hopkins Marine Station (HMS) and Naval Postgraduate School (NPS). (Depths in fathoms.)

III. INSTRUMENTATION

A. ELECTRONICS

Photomultiplier light detectors for at-sea bioluminescence studies were initially designed and developed independently by Clarke and his co-workers and by Boden and Kampa in the mid 1950's (Tett and Kelly, 1973). The basic circuit components are a photomultiplier tube, a regulated high voltage power supply and a recording or monitoring device. In this study a RCA 931A photomultiplier tube was used initially and later replaced by a 1P21 tube. The tubes are identical, except the 1P21 has a luminous sensitivity about four times greater. The 931A and 1P21 have a maximum spectral response at about 4000 Å (Figure 2). Thus bioluminescence (470nm to 500nm) occurs at the 70% to 83% relative sensitivity portion on the response curves of these detectors.

The high voltage power supply may be located on deck or in situ. A shipboard regulated power supply which could be adjusted manually in one-, ten-, or one hundred-volt steps up to 1200VDC was tried initially. This allowed the use of a much simpler in situ unit, as only the photomultiplier tube had to be encased in an underwater housing. However, this required a cable capable of carrying high voltages (1000-1200 VDC) to depths of several hundred meters.

Later the underwater housing was enlarged, and a high voltage battery pack was made from four 300 VDC batteries. A small operational amplifier was placed in the underwater

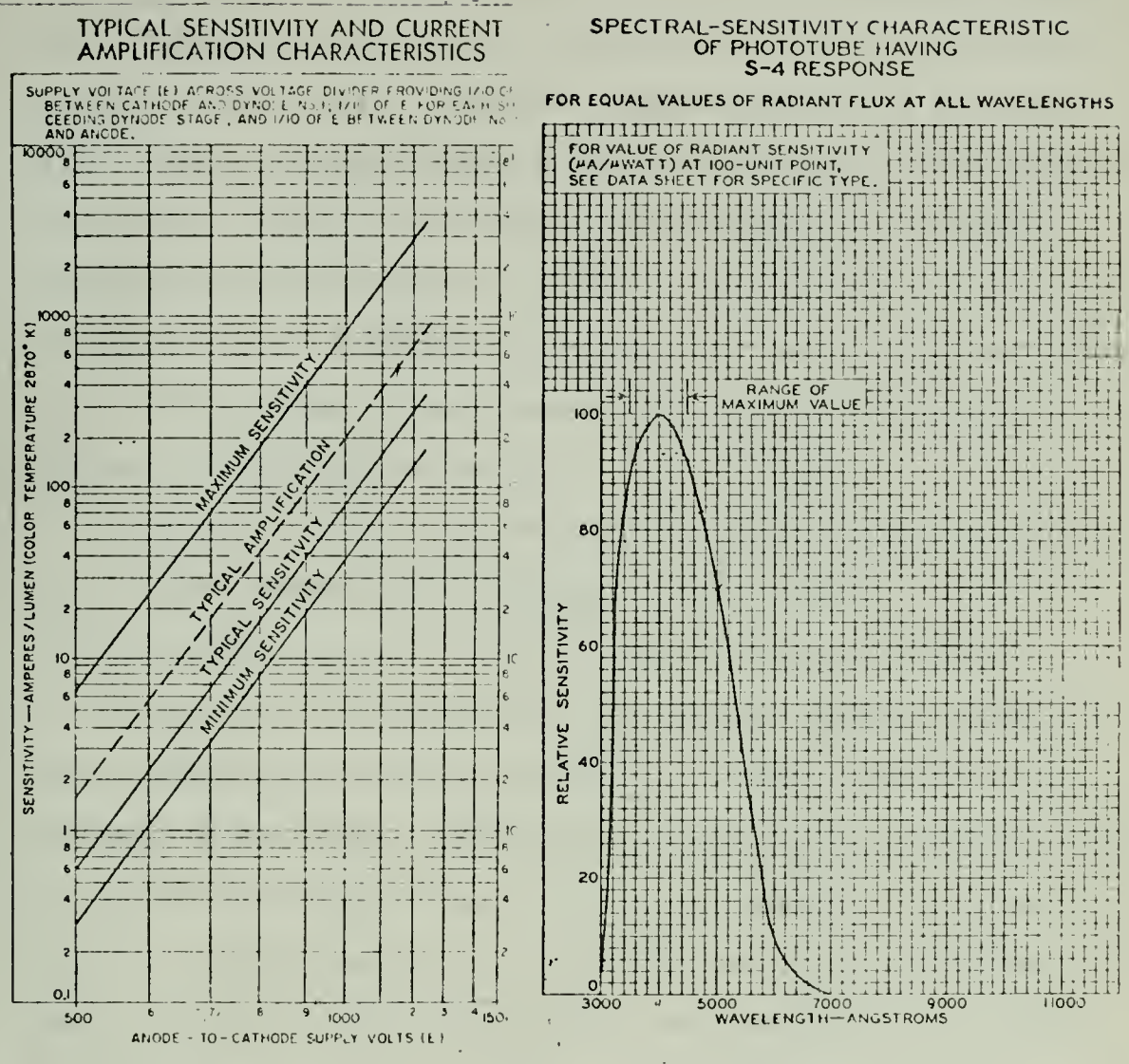

Figure 2. Spectral sensitivity characteristics and typical sensitivity and current amplification characteristics of 1P21 phototube. (From RCA data sheets 92CM-6152R9 and 92CM-6454R5, respectively.

housing along with the battery and a deck controlled on-off relay to apply the high voltage across the photomultiplier tube (Figure 3). The deck units consisted of an attenuator and zero offset unit, a Hewlett Packard model 680 five-inch strip chart recorder having a frequency response of one-half second full scale, and a small DC power supply to operate the on-off relay (Figure 4). The in situ detector unit was suspended on R/V ACANIA's four-conductor armored hydrographic cable. Slip rings allowed measurements to be performed while raising or lowering the sensor.

Calibration of the photomultiplier tube was accomplished with a Gamma Model 220-1A standard light source of 100 in a photographic dark room. The sensor was placed at a distance of 57 inches from a standard source rated at a color temperature of 2854 ± 50°K and at 100 footcandles at a distance of one foot. The spectral-sensitivity characteristics of the 1P21 photomultiplier tube (S-4 response) for a radiant flux from a tungsten source at 2870°K are given in Appendix D. This peaks at about 5100 Å, fairly near the 4700 to 5000 Å for bioluminescence in the ocean. At a distance of 57 inches the amount of light from the source is reduced to 4.43 footcandles.

Neutral density filters were used in steps of 1.0 to reduce the light to the sensor. The high voltage to the 1P21 tube was increased in 100 VDC steps from 800 VDC to 1200 VDC. The calibration curves are depicted in Appendix E. The signal in millivolts to the recorder (Hewlett Packard Model 680)

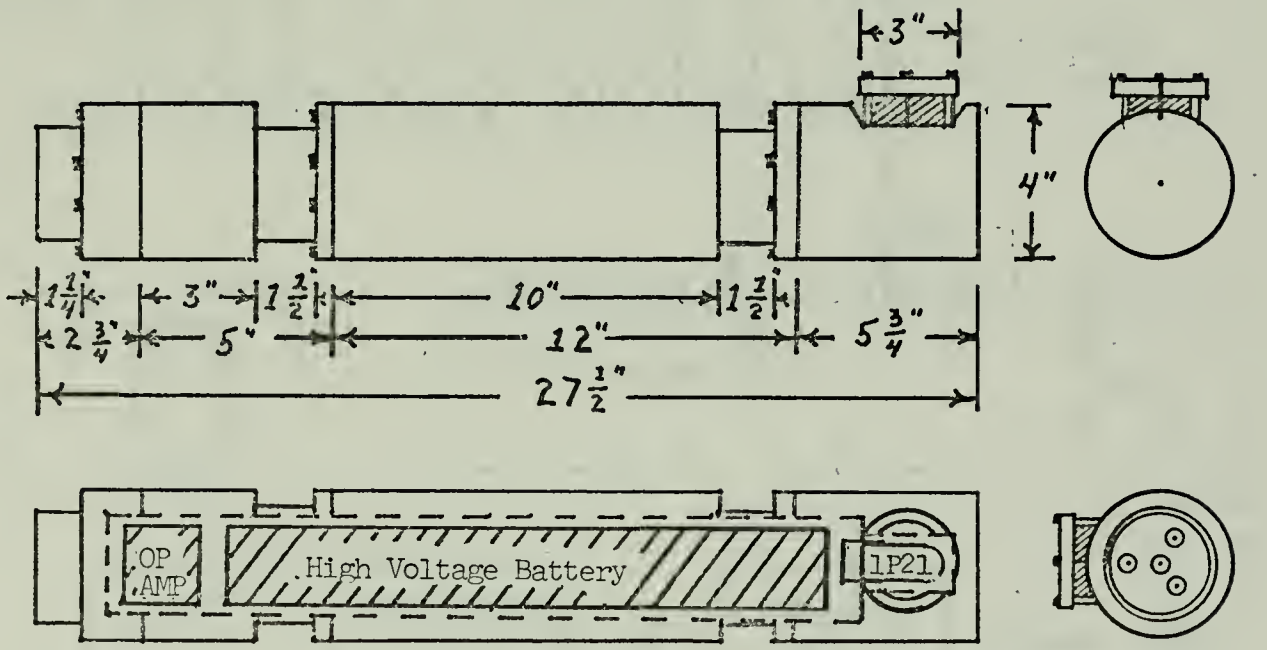

Figure 3. Photomultiplier tube in underwater housing.

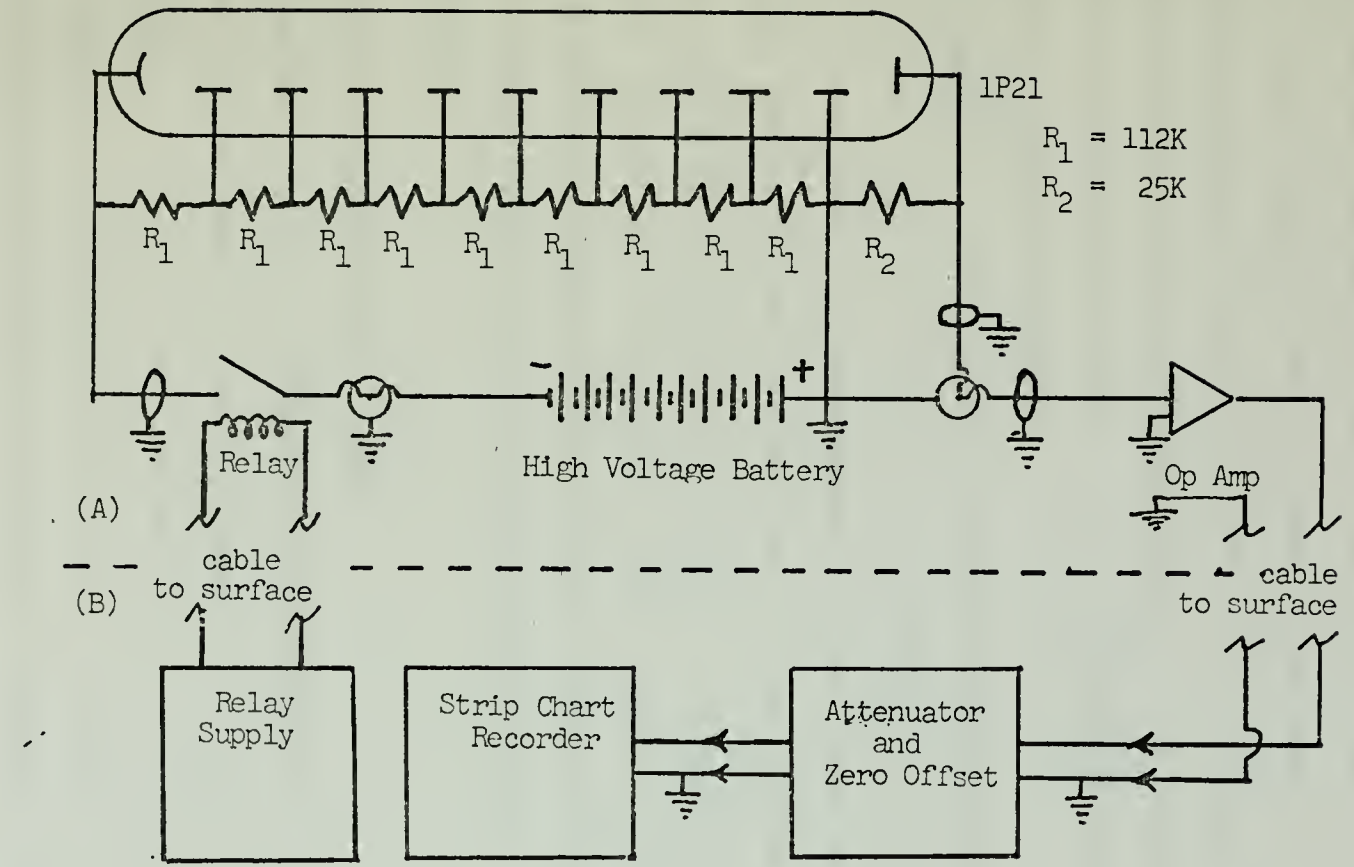

Figure 4. Circuit for photomultiplier light detector showing subsurface (A) and surface (B) units.

is plotted versus the illumination at the window of the detector in footcandles.

No corrections were made to take into account the fact that the Fresnel reflection coefficient for normal incidence for the air-plexiglass window surface of the detector as used during calibration and laboratory work differs from that for water-plexiglass surface during the in situ measurement. For the air-plexiglass surface, $r_{AP} = \left(\frac{n_P - n_A}{n_P + n_A}\right)^2$, and for the water-plexiglass surface, $r_{WP} = \left(\frac{n_P - n_W}{n_P + n_W}\right)^2$, where $n_P = 1.49$ is the refractive index for plexiglass, $n_P = 1.34$ is that for seawater, and $n_A = 1$ is that for air, all for the sodium D lines. Thus, since the calibration was performed in air, the in situ recorder readings should be multiplied by a factor $\frac{1}{1 - (r_{AP} - r_{WP})}$ = 1.038 to obtain the true in-situ light values. In the present study no attempt has been made to make this correction as it is small compared with the other experimental uncertainties.

B. VERTICAL MIGRATION TUBE (VMT)

The major problems of artificial stimulation of the organisms in the ocean by the equipment, the unknown distance from sensor to organism, and the inability to identify the exact organism or oganisms emitting light was approached by designing and constructing an underwater retaining tube. This tube holds the organism (or organisms) being tested at a known distance with minimal mechanical stimulation.

Designated as a vertical migration tube (VMT) it was designed to contain small organisms of euphausiid size (12-25 mm length) within the field of view of the sensor and yet allow the organisms to swim freely within a restricted area (Figure 5). Access holes at either end of the tube allow pressure equalization, while temperature equalization occurs through the plexiglass.

The tube is suspended from the hydrographic cable with the photomultiplier light detector attached (Figure 5) and then lowered into the ocean. Light can be shut out from the VMT and the detector by placing a black plastic curtain around them. Retaining screens, which hold the organisms at any desired position along the VMT, allow free flow of water. Access to the VMT is through removable base plates. Two supporting rods of 3/8" aluminum allow a heavy stabilizing weight to be attached.

C. LABORATORY TEST EQUIPMENT

Initial attempts at laboratory testing of the bioluminescent light output of an individual euphausiid were indeterminate when methods previously developed (Tett, 1972) were used. This was due to the continually changing aspect and range presented to the sensor by the freely swimming euphausiid, the photophores (luminescent organs) of which are located laterally along its abdominal segments (Figure 6). When placed in a small beaker, stimulated by electronic flash (Hardy and Kay, 1964), and placed over the photomultiplier

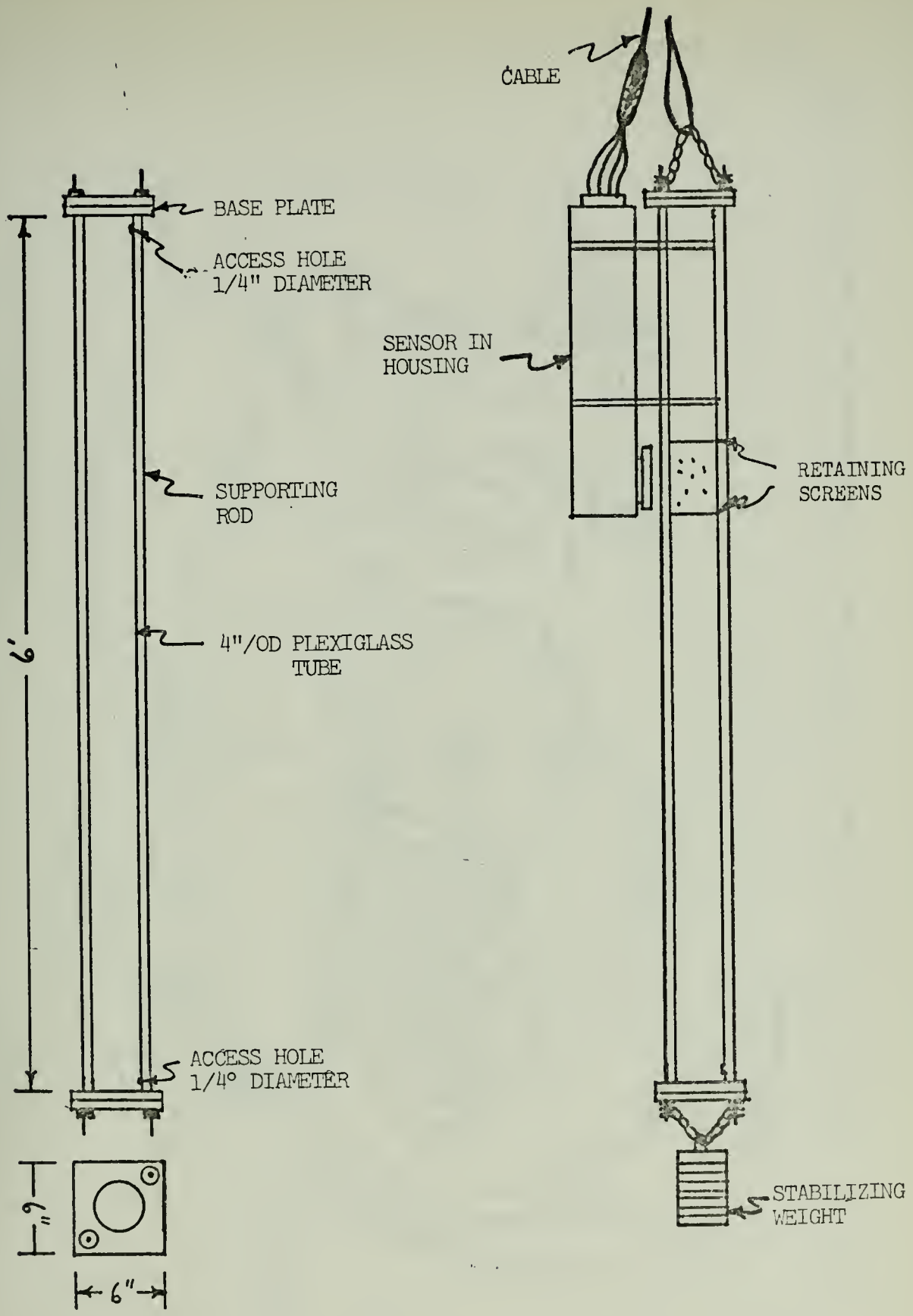

Figure 5. Vertic l Migration Tube with and without photomultiplier sensor attached.

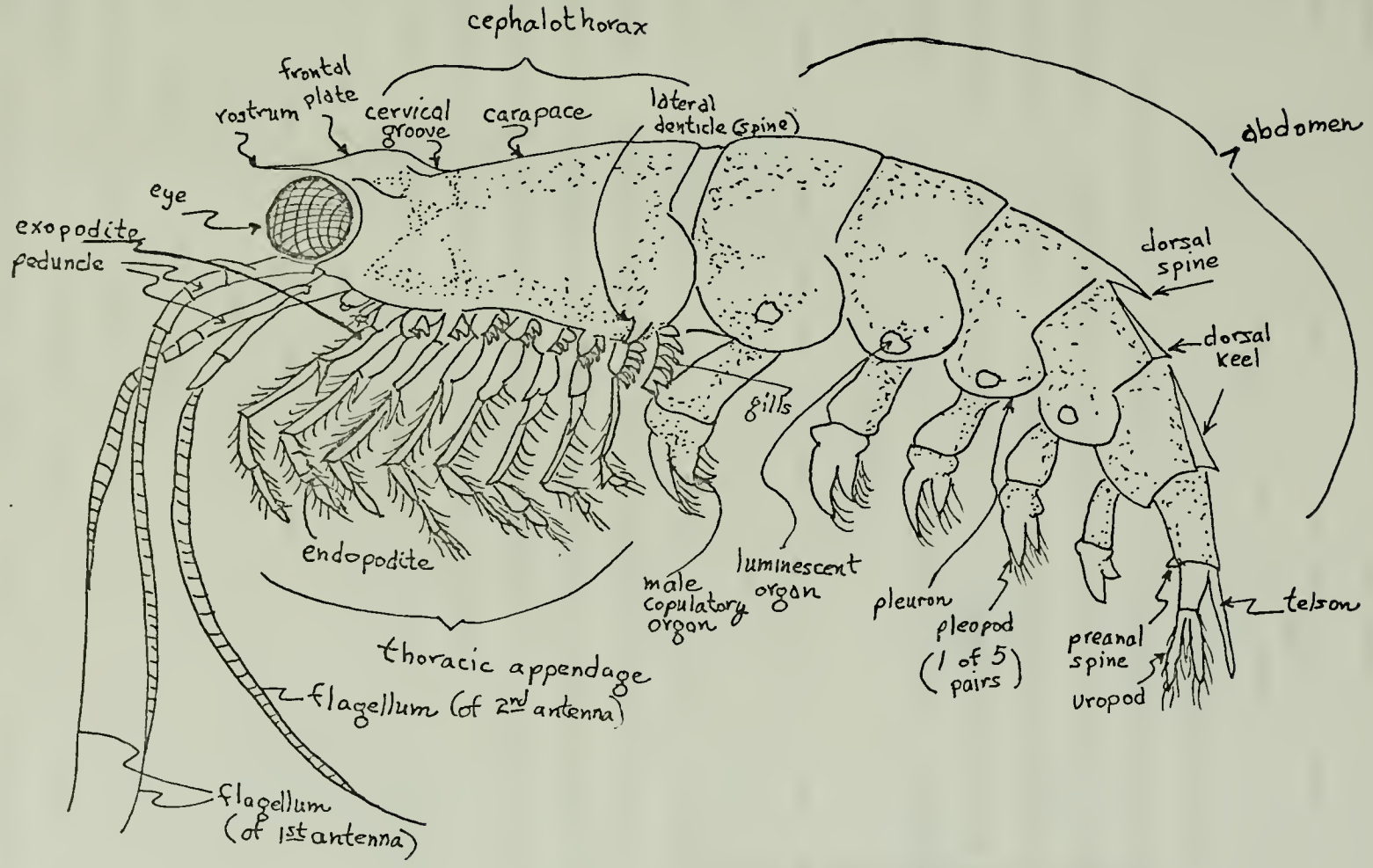

Figure 6. Euphausiid showing general terminology. (After Mauchline and Fisher, 1969)

sensor, the euphausiid swims in several different patterns. When monitored in darkness under an infrared scope, the euphausiid is seen to swim in axial somersaults within the water volume, in circles on the surface, up or down in an upright position, around the side of the beaker, or simply to rest on the bottom. Each of these orientations presents a different aspect of the animal's photophores to the sensor. Definitive quantitative measurements of such light output are exceedingly difficult to make. An attempt at a standard stimulation is also difficult as the euphausiid can have a different receiving aspect for the stimulation depending upon its instantaneous orientation.

New equipment and methods were developed to overcome these difficulties. The euphausiid had to be restrained with the least handling in order to prevent damage and behavioral changes. Since the sensor was unidirectional and the euphausiid possessed photophores on both sides of his body a specially silvered flashlight reflector was used to collect the light and direct it toward the detector.

The euphausiid is captured tail first in a small glass tube from its one-quart laboratory container (Figure 7a). It can then be easily induced to swim up into the test chamber made from a small Liebig condenser (Figure 7b). The inner chamber of the test tube is open both at the top and bottom, with the bottom drawn out into a much smaller diameter. The bottom inch of the tube forms the test chamber and is large enough to allow the euphausiid some movement but not large enough to allow it to turn end for end. The

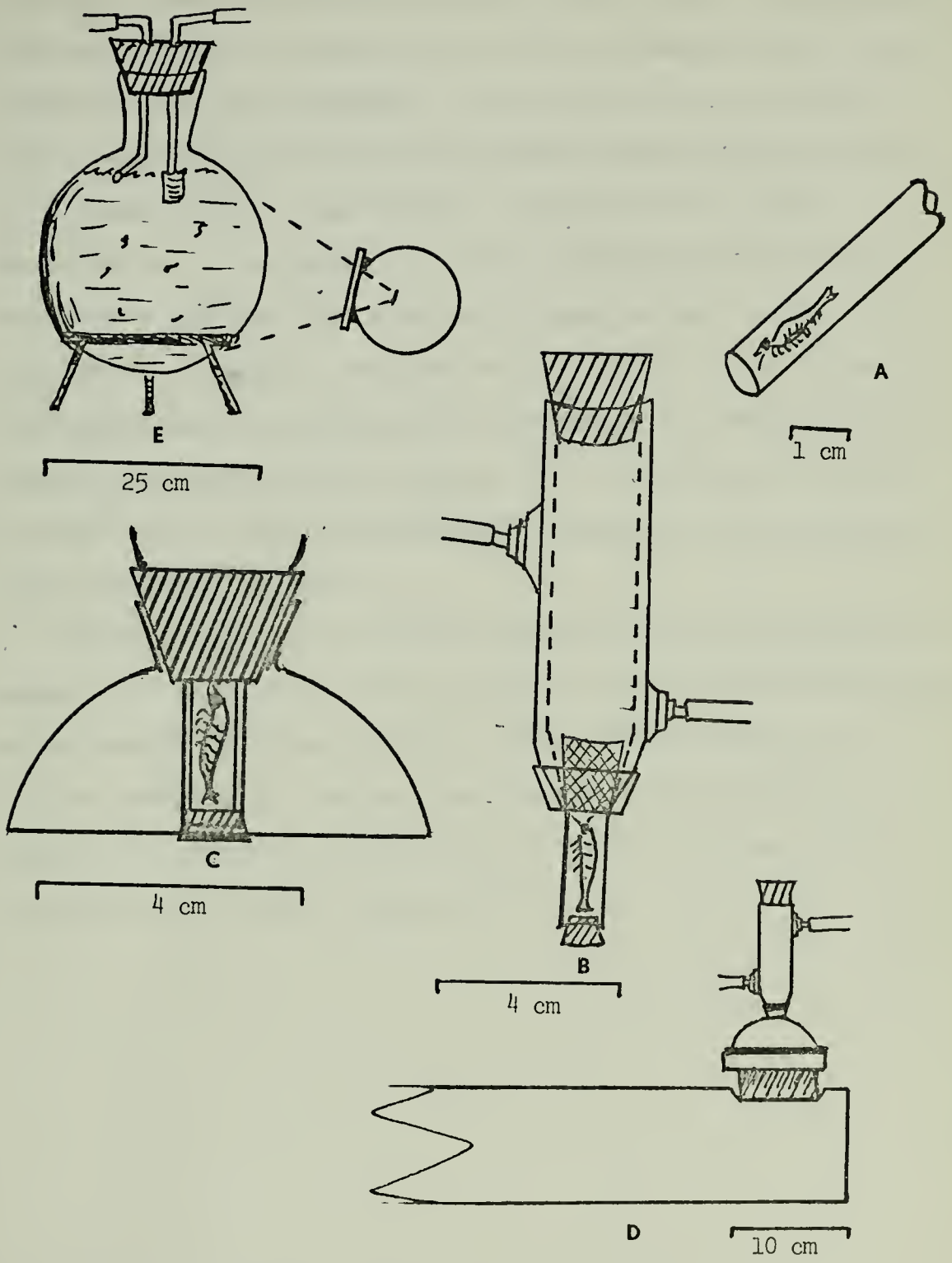

Figure 7. Laboratory test apparatus showing sequence followed in testing euphausiids. A. Euphausiid in tube. B. Euphausiid in test chamber. C. Test chamber with euphausiid in reflector. D. Apparatus in position. E. Spontaneous test.

euphausiid is retained in position by a screen at the top of the test chamber which allows water and oxygen exchange with the larger water volume above, and by a rubber cork at the bottom of the test chamber. The outer chamber is sealed except for two hose connections which allow a flow of water to maintain water temperature. The euphausiid, after swimming into the chamber, is flash stimulated from directly above at a distance of 10 cm and placed at the focus of the reflector, a position determined by a rubber stop (Figure 7c). The apparatus is immediately placed above the photomultiplier sensor, as can be seen in Figure 7d. The reflector directs a large part of the bioluminescent output of the euphausiid onto the light detector.

Tests of spontaneous bioluminescence were conducted in a large 3000 ml Florence flask equipped with an adjustable-flow water supply in order to effect temperature control and oxygen stability. This was monitored by positioning the sensor at a distance which would allow it to accept light from the entire flask (Figure 7e).

IV. PROCEDURES, TESTS AND RESULTS

A. AT SEA

1. General Procedures

The Naval Postgraduate School's research vessel ACANIA was used for the at-sea portions of this research. Typical cruises were of seven hours duration (1630 to 2330) to cover the period of upward vertical migration of the DSL. Travel time to station was about 80 minutes including a ten minute stop at CalCOFI* station 1 (Figure 1) for a vertical plankton haul to 30 meters with a one-meter net and an XBT** cast as part of another study.

A 12 KHz fathometer record was made during transits and while on station. The fixed operating frequency prevented good DSL resolution, and the fathometer was used primarily to aid in station keeping. A surface temperature and an XBT were taken upon arrival at CalCOFI station 3. Testing was accomplished according to the procedures described in the testing section below and net hauls were made. A one-meter net with a .75 mm mesh size was used to make vertical plankton hauls to 100 meters. Collections of E. pacifica and other DSL organisms were made after dark when most of the DSL vertical migration had taken place. This insured good collections with minimal damage to the organisms (Komaki, 1966).

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*California Cooperative Oceanic Fisheries Investigations

**Expendable bathythermograph

A one-quart glass jar was used for the cod end of the net. Once aboard, the catch was poured gently into a flat sorting pan. Species identification was performed utilizing a taxonomic key based on descriptions of the seven local species (Mauchline and Fisher, 1969). Initial identifying features for E. pacifica (Figure 6) are hemispherical eye shape, lack of a rostrum, lack of elongate thoracic legs, and a slender body shape. Final sorting was accomplished later in the laboratory. Transfer from pan to large plastic containers was performed with small plastic spoons and large bore (5-6 mm) glass tubes. The large containers were then placed in the ship's refrigerator in order to maintain them in a cold, dark environment until transportation to the laboratory. A second net haul to 100 meters was taken and preserved in a 5% formalin solution for later determination of euphausiid species present during the test period (Appendix B).

2. At-Sea Tests and Results

a. Equipment Checkout

Test procedures at sea began with an onboard checkout of the equipment and circuitry. The underwater housing containing the photomultiplier tube, battery, amplifier, and relay was pre-cooled in the refrigerator to lessen initial battery drift due to temperature changes. The housing was shielded from ambient light with black plastic and tape and lowered into the ocean for a dark cell

test. At a recorder setting of 5 mv full scale, dark current and battery drift were determined as functions of depth to 200 meters (Figure 8).

b. Ambient Light Measurements

After dark cell tests the sensor was returned to the surface, the light shield removed, and the unit lowered again into the ocean to record the bioluminescence of the natural environment. Penetration of skylight into the ocean prevented evaluation in the upper 25 meters. The high level of bioluminescence at 25 meters required a 50-mv full scale setting on the recorder to keep most of the activity on scale. The same setting was used at all depths. Five-minute tests were made at 25-meter intervals between 25 and 150 meters and at 50-meter intervals from 150 to 300 meters. Records were made at one and eight inches per minute at each depth to maximum depths of 250 to 300 meters. A continuous run to the same maximum depth was also made with the sensor stationary only at the bottom of the run. The bioluminescence of the environment decreased in flash frequency with increased depth, the greatest changes occuring in the upper 50 meters. This decrease in activity was most likely due to a decrease in numbers of bioluminescent organisms with increased depth. Results of this test made during ACANIA cruise 74-14 are depicted in Figures 9 and 10.

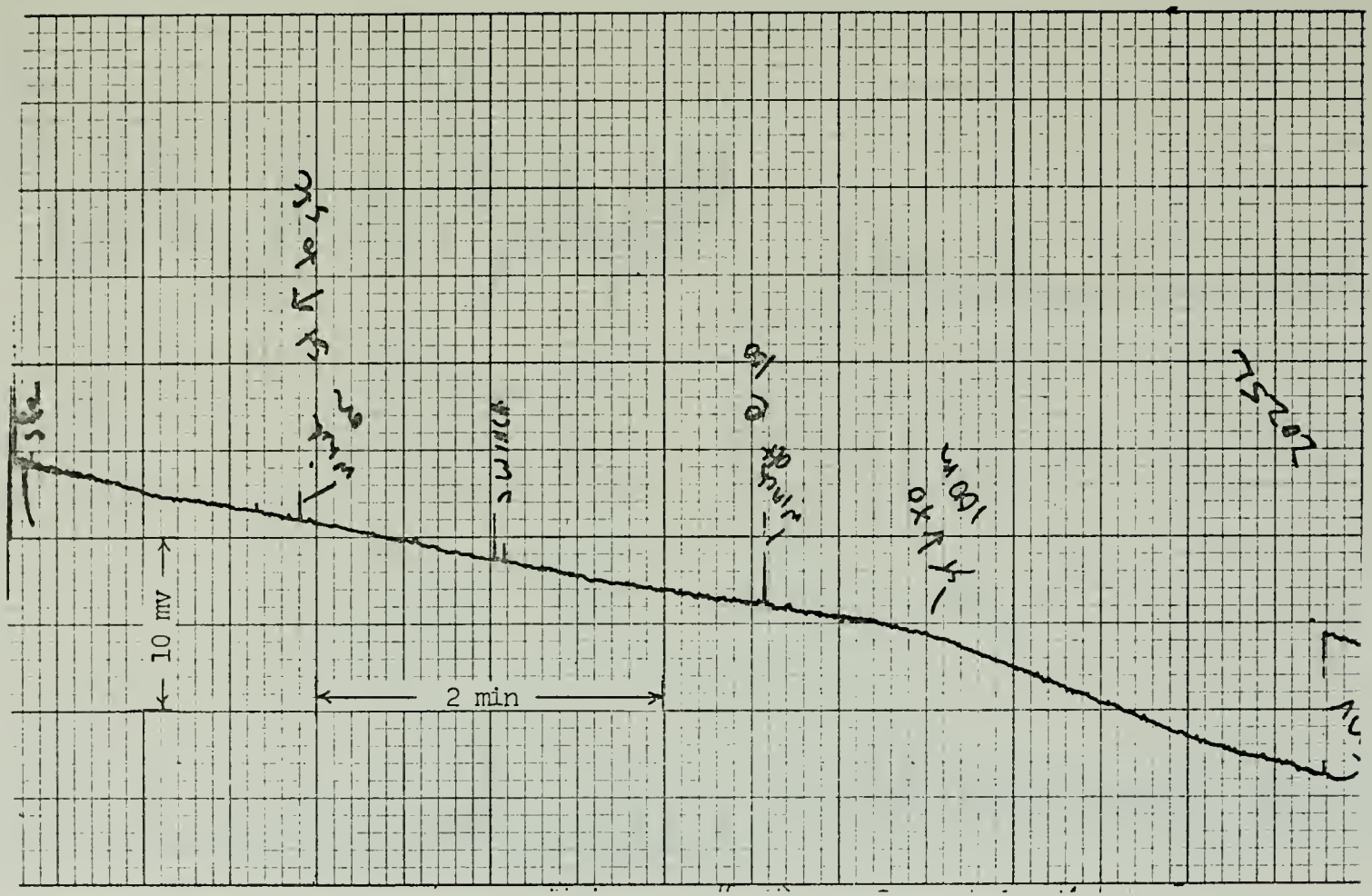


Figure 8. Chart recording of dark current of photomultiplier light detector at 100 meters depth.

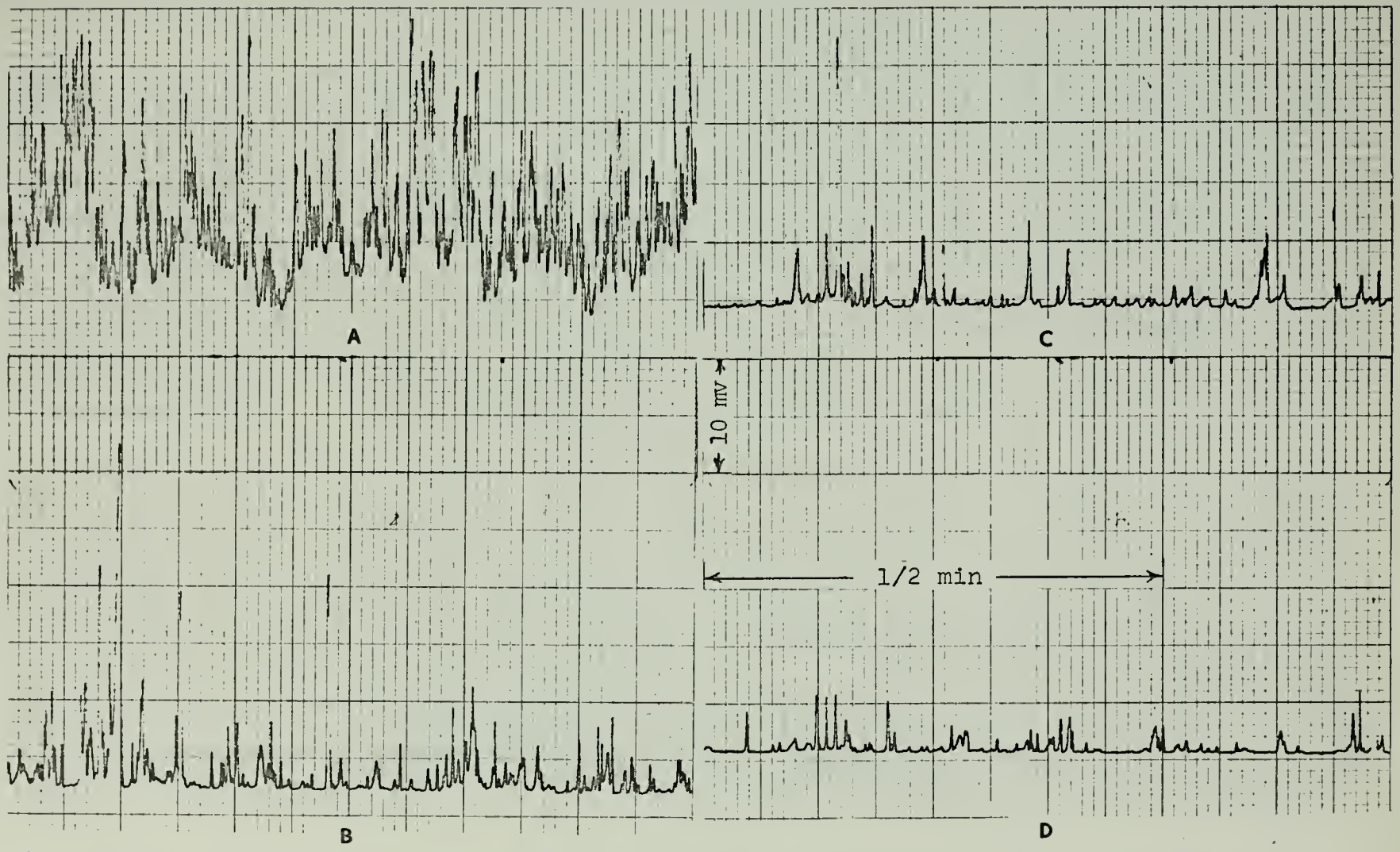


Figure 9. Bioluminescence of environment at 25 meters (A), 50 meters (B), 75 meters (C) and 100 meters (D). 10:00 PM.

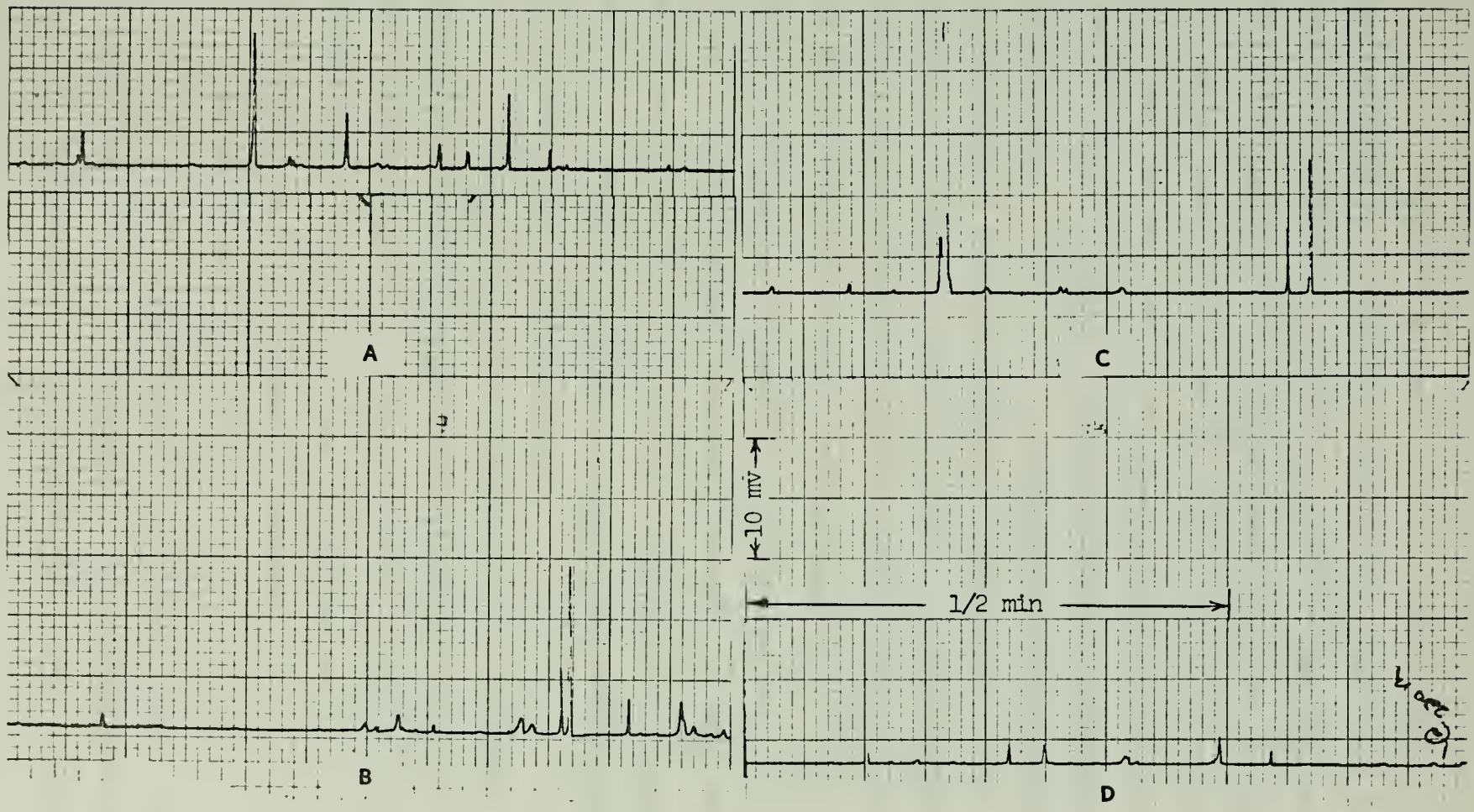


Figure 10. Bioluminescence of environment at 125 meters (A), 150 meters (B), 200 meters (C) and 250 meters (D). 10:00 PM

c. VMT with Euphausiids (Excluding Ambient Light)

Once the vertical profile of bioluminescence had been recorded to a predetermined depth, a net haul was made to collect organisms to test in the VMT. The VMT was attached to the electric hydrographic wire and the light detector to the VMT (Figure 5). The VMT was then carefully filled with surface water to prevent the formation of small bubbles, which can attach to the euphausiid appendages and hinder movement. The first retaining screen was positioned, ten euphausiids were introduced, and then the second screen placed into position. The end plate was then fastened into position, and the sensor and VMT partially wrapped and taped with black plastic to screen out ambient light. The unit was then lowered to 50 meters, where it was kept for 20-25 minutes to allow battery temperature compensation and to permit the euphausiids to become acclimatized. Stimulation due to handling and exposure to light will cause initial bioluminescence in the organisms.

The role of bioluminescence in the marine environment and how physical and environmental factors affect light output are largely unknown. Hardy and Kay (1964) and Tett (1972) have shown that mechanical agitation and bright light can stimulate bicluminescent activity in laboratory tests of euphausiids.

After acclimatization the initial tests were repeated with the sensor detecting only the bioluminescence of the ten euphausiids. The euphausiids were subjected

primarily to pressure and temperature changes in the water column. Other parameters could affect this activity however. There are comparatively large changes in oxygen, pH, carbon dioxide, salinity, and both phosphorus and nitrogen (in the form of nutrients) in the upper oceanic waters (Horne, 1969). It is felt that these changes were probably attenuated due to the small size of the 0.25-in diameter access holes, the relatively large internal volume of water within the VMT, and the distance from the access holes to the test area within the tube (Figure 5).

The bioluminescent record of the euphausiids was similar in profile to the record of environmental bioluminescence made an hour earlier on the same cruise. The flash frequency of bioluminescence again decreases with increased depth in the water column. Here, however, the euphausiids are physically restrained to the same area of the VMT and the decrease in bioluminescence must be due to less activity by the same number of organisms. The bioluminescent activity increased as the sensor and VMT were raised (Figure 11).

A second series of tests starting three hours earlier in the evening was conducted on ACANIA cruise 74-19. The bioluminescent activity of the environment did not show the marked decrease with depth evident on the first test. A continuous trace is used to depict this in Figure 12. It is felt that the increased bioluminescence at depth compared to that for the previous test (ACANIA cruise 74-14) was due to incomplete vertical migration of the DSLs due to the earlier

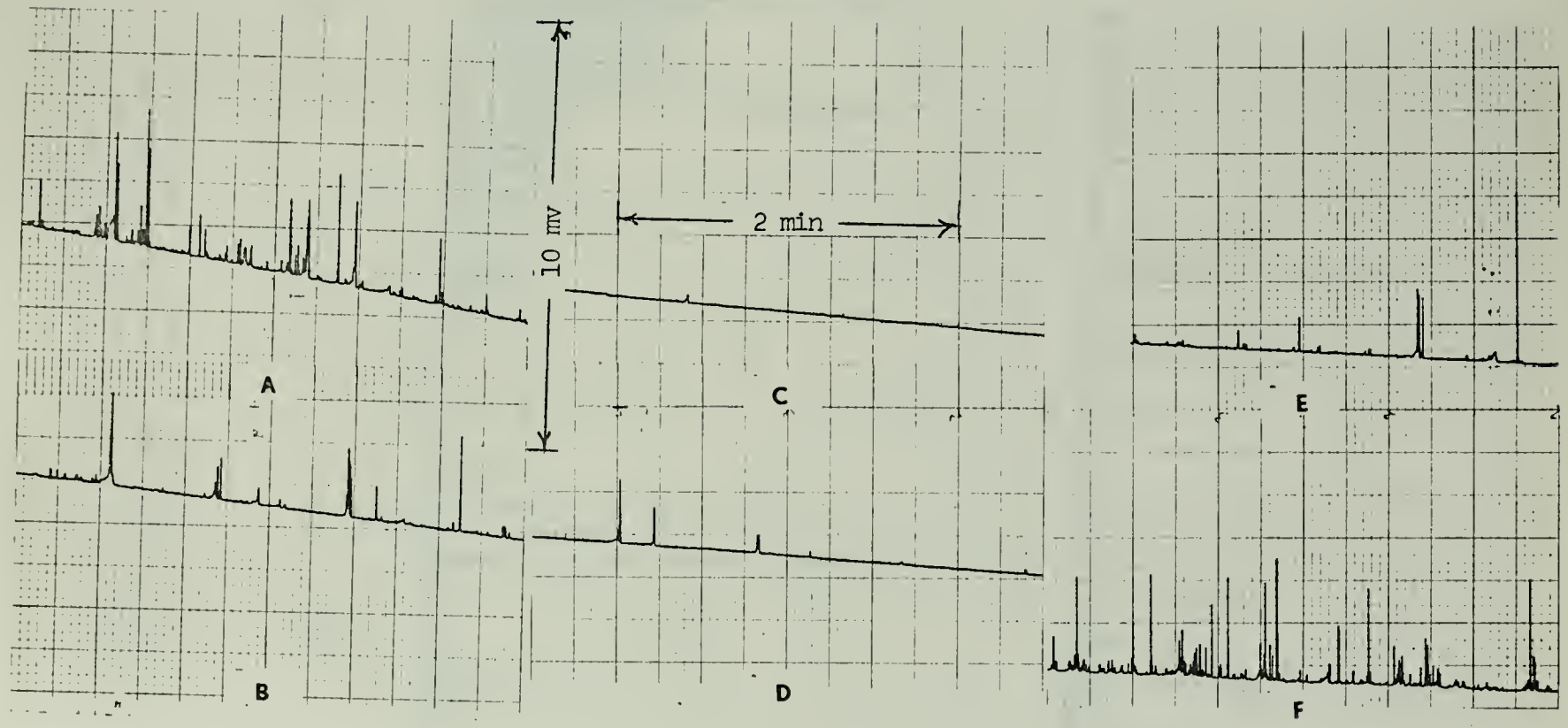


Figure 11. Bioluminescence of 10 euphausiids at 25 meters (A), 50 meters (B), 100 meters (C) and 150 meters (D); and at 50 meters (E) and 25 meters (F) on return trip to surface.

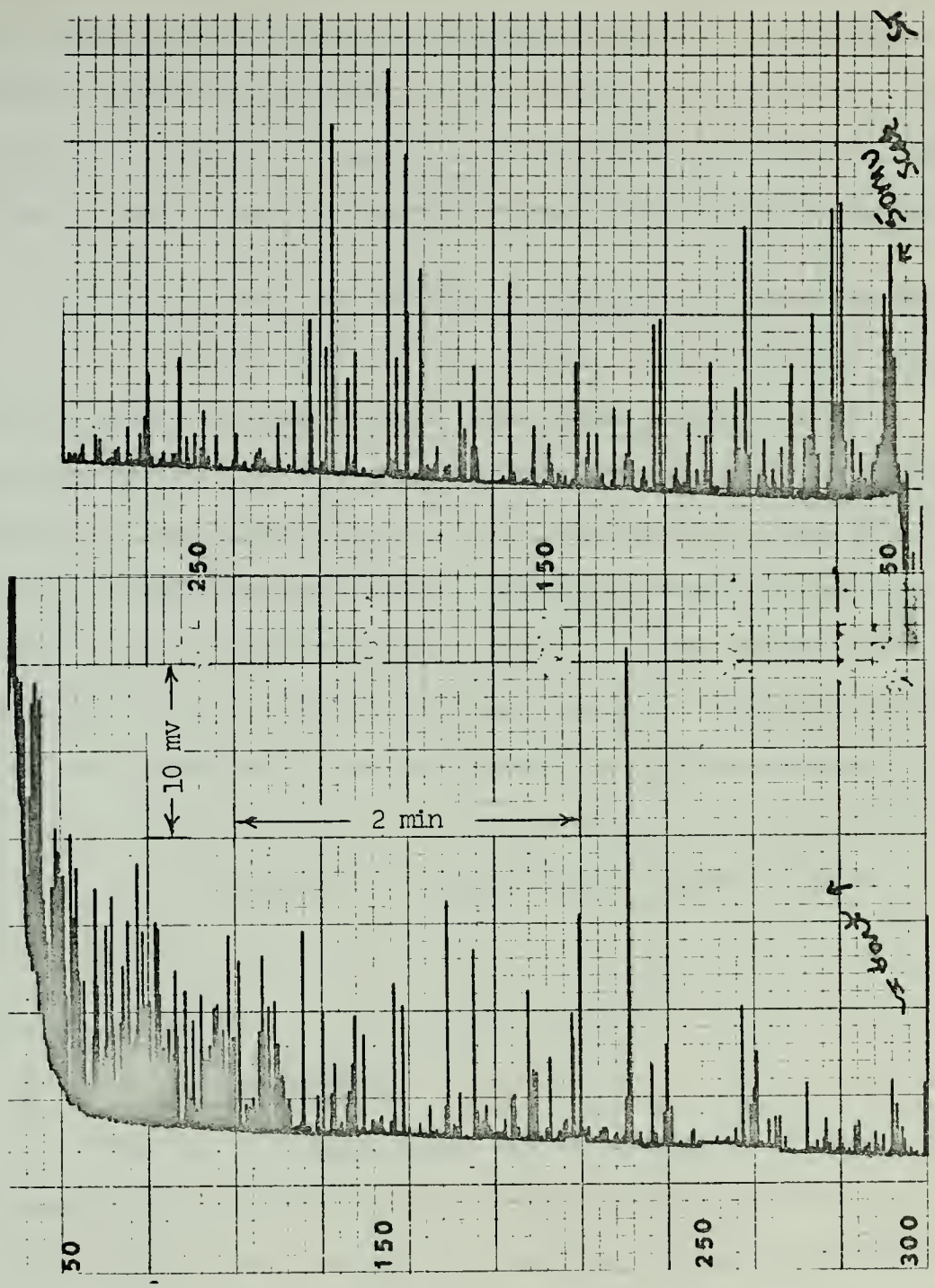


Figure 12. Continuous trace of bioluminescence of environment from 50 meters (top right) to 300 meters (lower right) and return to 50 meters (lower left). Read right to left. 8:00 PM.

hour of the test. This would imply that the organisms were still spread out vertically throughout more of the water column. Figure 13 depicts a record of ten euphausiids (tested as before on ACANIA cruise 74-14) from 50-meter to 150-meter depths. The frequency flash rate again decreased with depth.

d. VMT with Euphausiids (Including Ambient Light)

Another test of euphausiid bioluminescence was added on ACANIA cruise 74-19. The black plastic cover shielding the sensor and euphausiids from outside light was reduced in coverage to shield only the sensor's view of outside bioluminescence. The restrained euphausiids were exposed to other bioluminescence due to their wider field of view. This was to test for changes in bioluminescent activity of the test euphausiids when exposed to outside natural light stimulus. The test results showed increased bioluminescent activity in the presence of such outside light (Figures 13 and 14).

B. LABORATORY

1. General Procedures

Laboratory measurements and maintenance of the euphausiids were carried out at Hopkins Marine Station of Stanford University, located in Pacific Grove, California. The laboratory was supplied with two sources of filtered sea water, one at the ambient ocean surface temperature of about 12°C, and a second which could be temperature controlled in

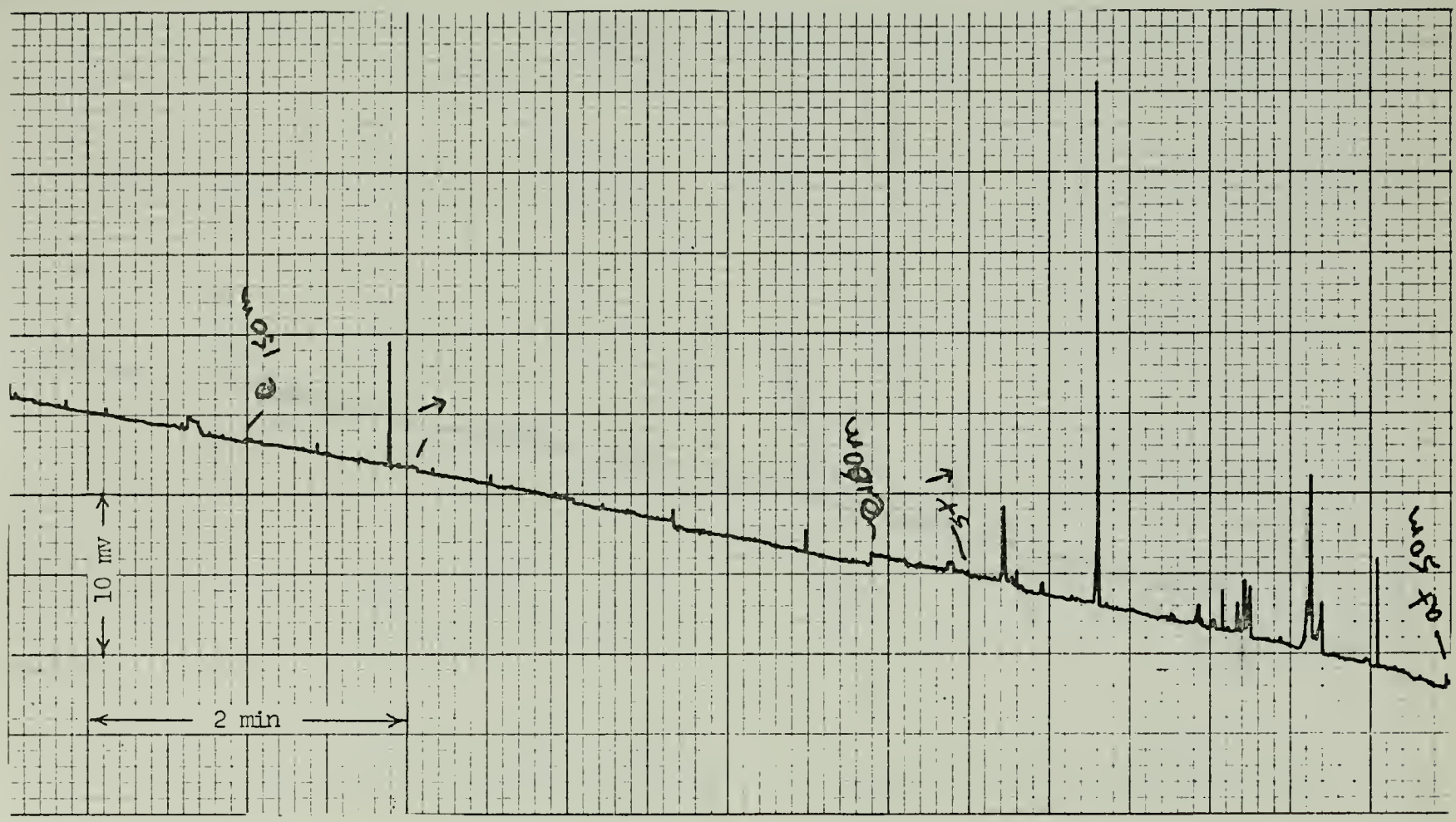


Figure 13. Continuous trace of 10 euphausiids from 50 meters (right side) to 150 meters (left side). Not exposed to outside light. 9:00 PM.

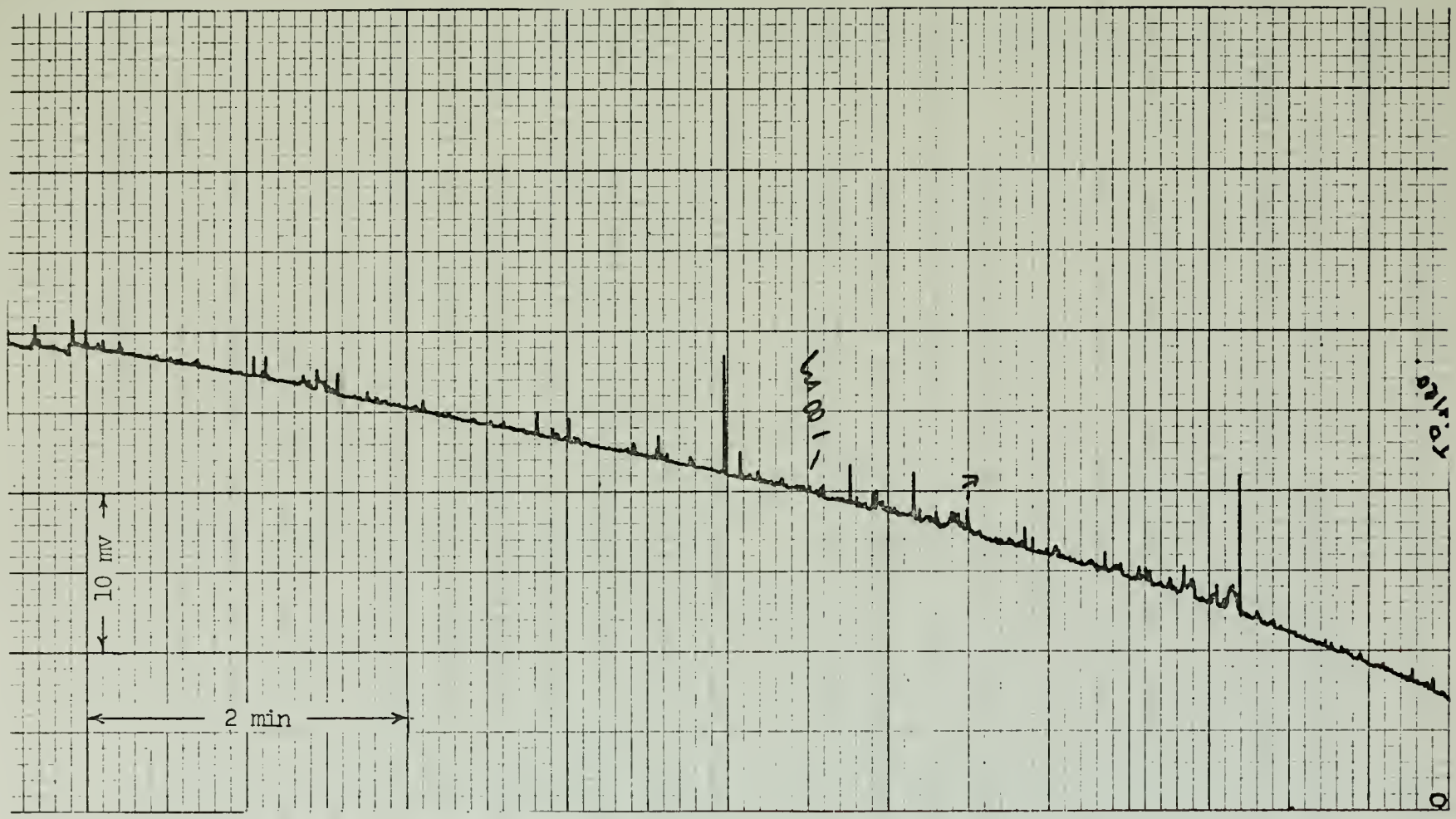


Figure 14. Continuous trace of 10 euphausiids from 50 meters to 125 meters (right to left). Exposed to outside light. 9:30 PM.

the laboratory and which was maintained at about 6°C. The laboratory was windowless and could be kept in complete darkness.

Following cruises the euphausiids were left overnight in large polyethelene containers placed in a water bath for temperature control. The next day the most active of the euphausiids were placed in individual one-quart polyethelene containers and maintained in either a 12°C or a 6°C water bath. Temperature control in the laboratory was to within ±1°C but temporary breakdowns caused some loss of euphausiids and occasional interruption of tests. Water in the one-quart containers was changed daily, and each euphausiid was fed about 100 Artemia salina nauplii daily (Lasker and Theilacker, 1965).

The photomultiplier light detector and associated units used in the laboratory were the same ones used at sea, except for the battery which was replaced by the adjustable high voltage DC power supply. Laboratory tests with this equipment were conducted in darkness except for a small microscope light having a red filter, which was used in monitoring the recorder base level. This light was shielded from the light detector during tests. Laboratory runs were made on consecutive days except during equipment breakdowns, laboratory water temperature failures, or when interrupted for the sea cruises, or the spontaneous bioluminescence tests which lasted up to 48 hours.

2. Laboratory Tests and Results

a. Spontaneous Activity Tests

These tests were designed to record any spontaneous bioluminescent activity by the euphausiids. They were set up immediately after a cruise (except when starting time was varied as a control), usually about 0100. Previous results with other euphausiid species (Mauchline, 1960; Tett, 1972) indicated that this type of activity would decline quickly after capture. The laboratory setup (Figure 7c) was shielded by a black plastic hood in the dark test room. The runs lasted from 10 to 48 hours.

Five groups of 15 euphausiids each were tested. Three different water temperatures were used. Two groups of 15 were tested at 12°C, two at 7°C and one with an alternating 7°C daytime temperature and 12°C nighttime temperature. Fifteen euphausiids were used, since none were pre-tested for bioluminescent activity, and experience had shown that only about one half of a tested group were active.

Group one, for which the temperature was alternated, was tested for 48 hours and showed repetitive activity between 0100 and 0400 hours and between 1830 and 2000 hours and repetitive inactivity at five times (Figure 15). Groups two and three (7°C) were run for periods of 14 and 10 hours respectively. Comparison of the records of these two runs indicated similar active periods between 0000 and 0130 hours and between 0400 and 0900 hours. No inactive matching periods were present in the time tested. Groups

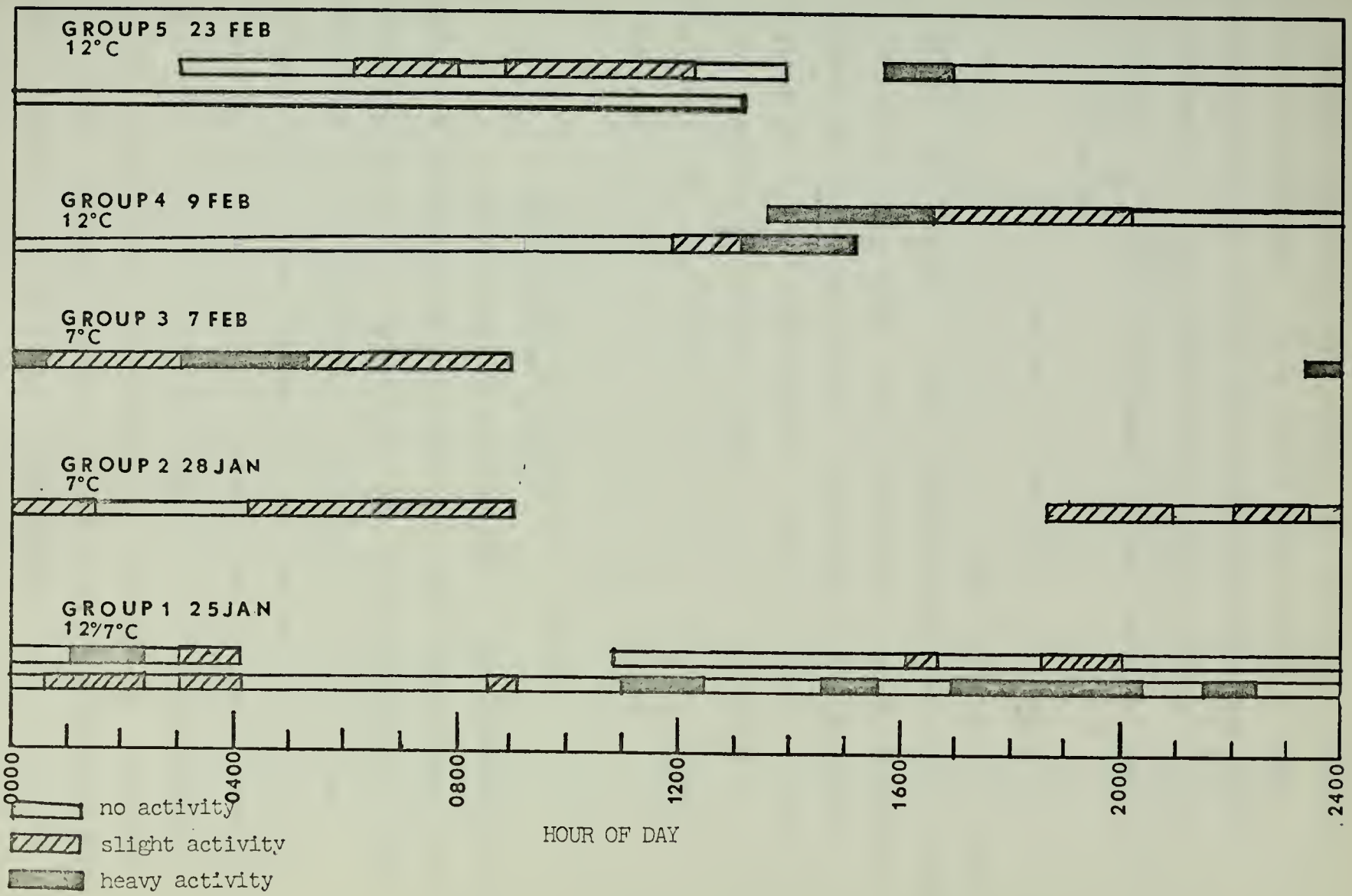


Figure 15. Spontaneous bioluminescence test results.

four and five (12°C) were tested for periods of 25 and 34 hours respectively. They showed more variation (less similarity) and more inactive time than the other groups (Figure 15). They matched activity only between 1530 and 1700 hours. Group four showed internal matching from 1500 to 1700 hours.

In evaluating the records no attempt was made to count the individual flashes, as low amplitude and slow chart speed (four inches per hour) compressed the individual flashes together on the record. The results were evaluated subjectively as "heavy activity" if the flashes were compressed into a solid record (Figure 16), "light activity" if the base line was evident between flashes, and "no activity" if only the base line was evident. The results were inconclusive when evaluated for a 12 or 24 hour cyclic rhythm.

b. Stimulated Bioluminescent Activity Versus Ambient Light

Stimulation of euphausiids in the laboratory to excite a bioluminescent response has been accomplished most successfully with a photoflash unit (Boden and Kampa, 1964). This method was adopted as a standard stimulus for the present study. The euphausiids were tested individually by removing each in turn from its one-quart laboratory container, placing it in a 60-ml glass beaker to which had been added 40 ml of water from the laboratory container, and flashing the beaker with the photoflash unit at a 10-cm distance when the euphausiid approached the side nearest the flash unit.

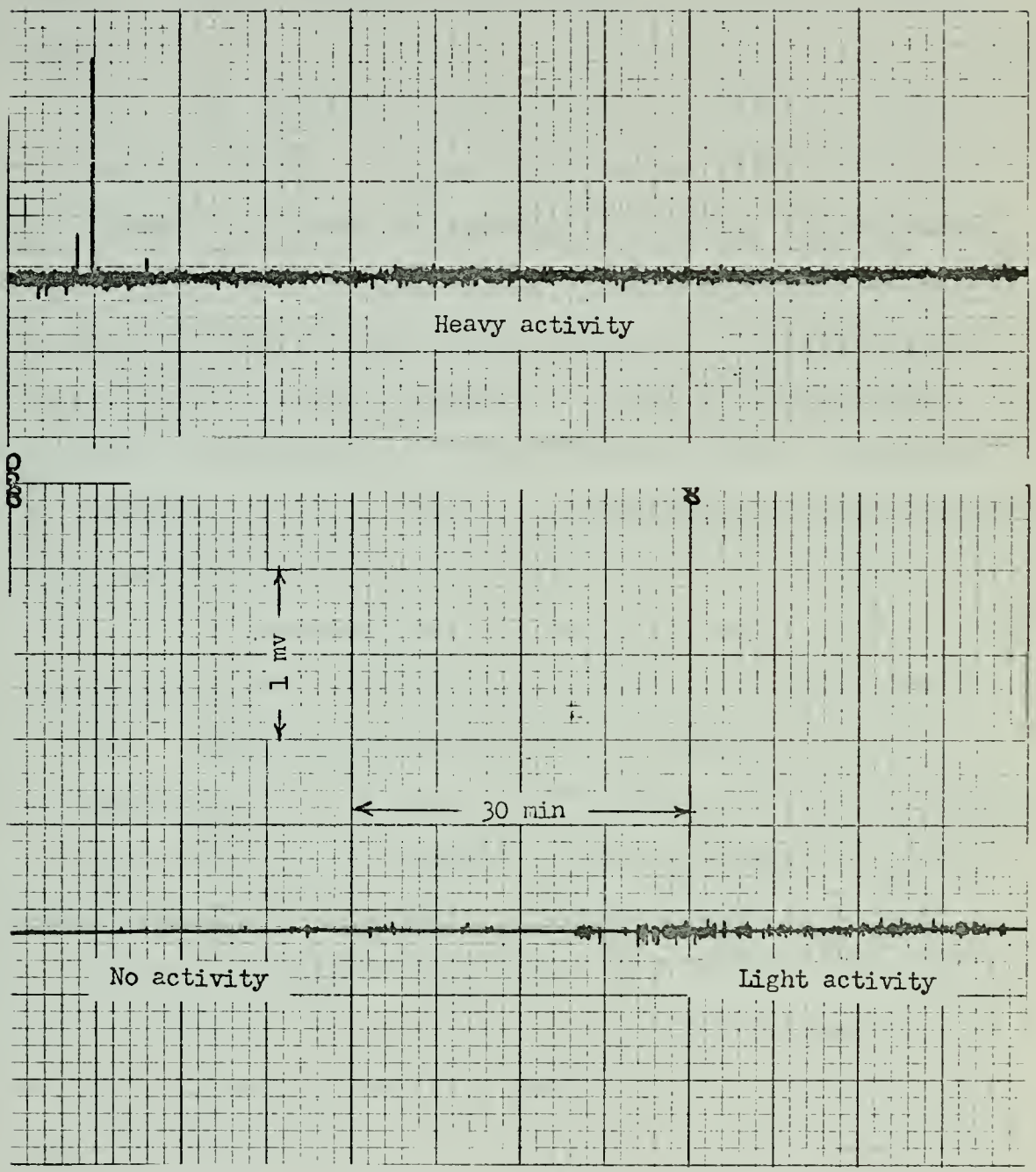


Figure 16. Example of spontaneous activity of 15 euphausiids

The beaker was then immediately placed over the photomultiplier sensor, which was shielded from outside light by placing a black cup over the beaker and sensor; the room light was turned off; and high voltage was applied to the sensor.

An initial qualitative test of bioluminescent activity of groups of euphausiids kept in different light environments was made to determine if ambient light levels would affect their light output. This also served as a test for the laboratory methods and resulted in the development of new procedures and equipment. It was felt after this test that techniques used up to this point were inadequate (see Chapter III.C) to evaluate quantitatively this parameter (Hardy and Kay, 1964; Tett, 1972).

A two-week test was run with twenty-five euphausiids which had responded positively to photoflash stimulus. They were divided into four groups as follows: ten euphausiids were placed in a completely dark environment, five were placed in a "bright" light environment (a 60 W, 1000-hour bulb at two feet), five were placed in a "dim" environment (estimated to be about 10^{-3} $\mu W/cm^2$ -- measured with a 931A phototube which was not calibrated absolutely, but was compared for relative sensitivity to the 1P21), and five were placed in a 12 hour alternating "dark" (night) and "dim" (daytime) environment. The ten euphausiids in the "dark" environment were tested once each day at 1800 and

also checked for the presence of moults. The other three groups were tested once every third day at 1700.

Subjective analysis of the records indicated that the bright light may have affected bioluminescent activity of that group as its bioluminescence fell off more quickly with time than that of the other three groups. There were no observable differences among the other three. The bright light group also had a higher mortality rate, i.e. all were dead at the end of two weeks versus a total of six dead out of twenty for the other three groups. A low light level environment was adopted for maintaining euphausiids for future tests. This was convenient for laboratory work and did not require abrupt light changes for feeding and change of water. No obvious relationship between moulting and bioluminescent activity was noted.

c. Stimulated Bioluminescent Activity as a Function of Temperature, Moulting and Time of Day

Tests based upon the experience gained from the previous ones were set up to check bioluminescent activity against two temperature controls, time of day, and moulting. Three groups of five euphausiids each were established after an initial test for positive bioluminescent response to photoflash stimulation. Euphausiids not bioluminescing within a four-minute time lapse after stimulation were not used.

Group one (euphausiids #1-#5) were kept in a 12°C constant temperature bath, group two (euphausiids

#6-#10) were kept in baths which were alternated from 7°C to 12°C and from 12°C to 7°C at intervals of about 12 hours; and group three (euphausiids #11-#15) were kept in a 7°C bath. Group two was changed from 7°C to 12°C water just prior to testing at midnight and from 12°C to 7°C water just prior to testing at noon. Tests were made daily from 1000-1400 and 2200-0200 for periods of up to 23 days. Moults found, time of test, water temperature, and an indication of bioluminescent activity were noted for each euphausiid tested. A six-minute record of the bioluminescent activity was made to determine light amplitude, flash frequency, total light output and reaction time from stimulus to start of bioluminescent activity (Table I). All groups were kept in a low light level environment with no disturbance except that resulting from testing.

Earlier tests made as part of the present study indicated that a six-minute record of the activity of the stimulated euphausiids was long enough to yield a good indication of the bioluminescent activity of the organism and not so long as to make the testing of fifteen euphausiids unmanageable. Some euphausiids displayed activity for as long as seventeen minutes, some for as few as two minutes. Eight minutes was the average period of activity. Figure 17 is an example of complete records of stimulated euphausiids which also illustrate these differences in activity.

During each test-period euphausiids were taken, one at a time and in their individual containers, to a

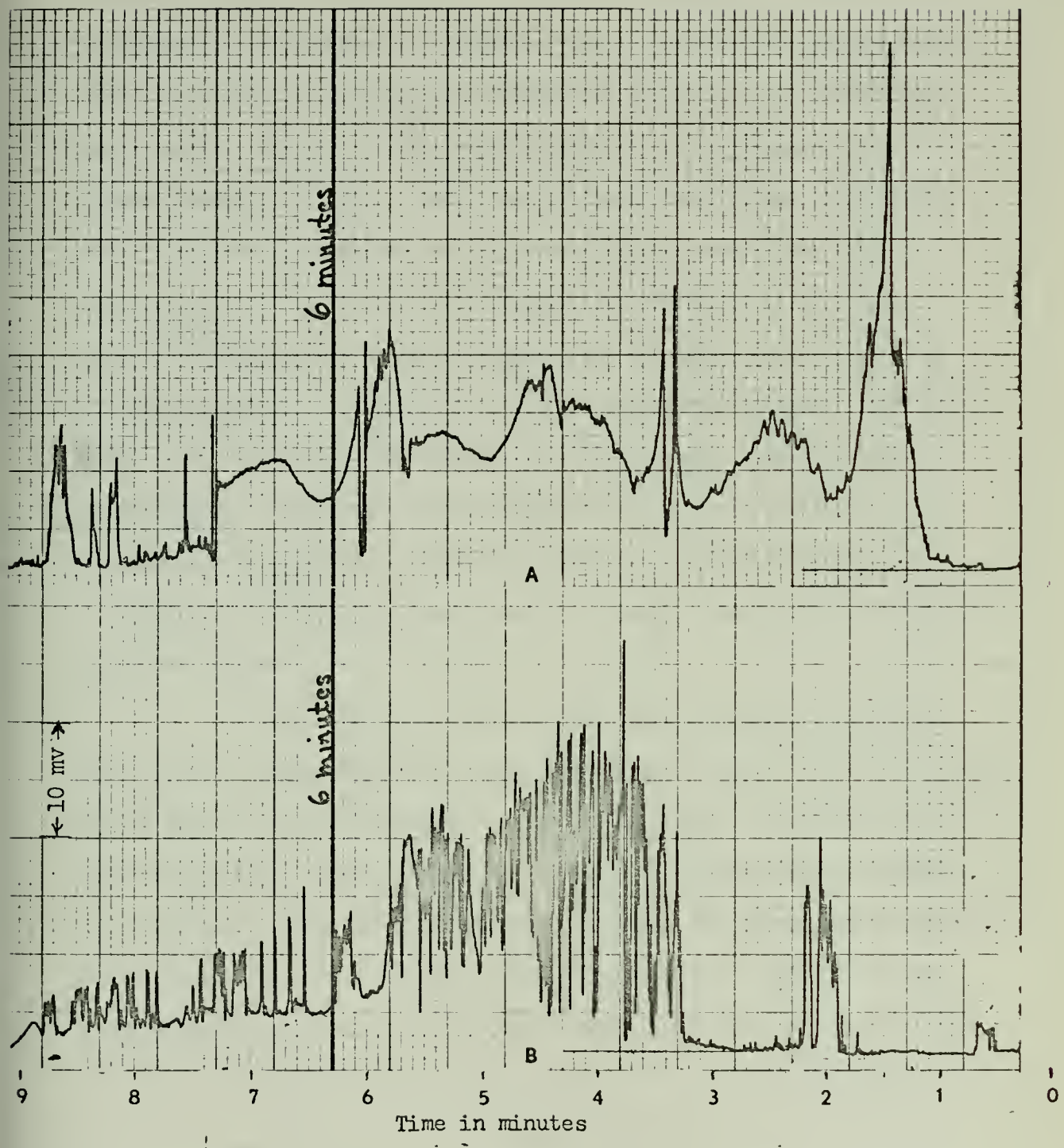


Figure 17. Example of complete record (A) of stimulated euphausiid, and example of almost (actually active for 2 more minutes) complete record (B) of stimulated euphausiid.

separate dark room. A glass tube was used to capture and transfer each euphausiid to the test chamber following the procedures described in Chapter III. The Bauer photoflash unit was used to deliver a standard stimulus by flashing the dorsal aspect of the euphausiid at a distance of approximately 10 cm. The test chamber was then inserted into the reflector which was positioned over the sensor aperture. A rubber seal between the reflector and the sensor housing and between the test chamber and reflector guarded against stray light. A black plastic hood was also used over the test apparatus, and the room lights were turned off. Because the average time from application of stimulus to the start of recording was 15-20 seconds, a 0.3-minute correction was added to each recorded test start time in Table I.

A 50-mv full scale recorder setting was used. This was a compromise between a more sensitive 5-mv setting, which would have detected lower level light activity, and a 100-mv setting which would have kept all readings on scale. A complete series of recordings for one specimen is displayed in Figures 18 and 19. The records show the change in the activity of the euphausiid from test to test and illustrate the type of laboratory record made for each euphausiid.

The record for each euphausiid was evaluated to determine total light output, flash frequency, maximum amplitude and reaction time. The total light output (area under each curve) was evaluated with a compensating polar

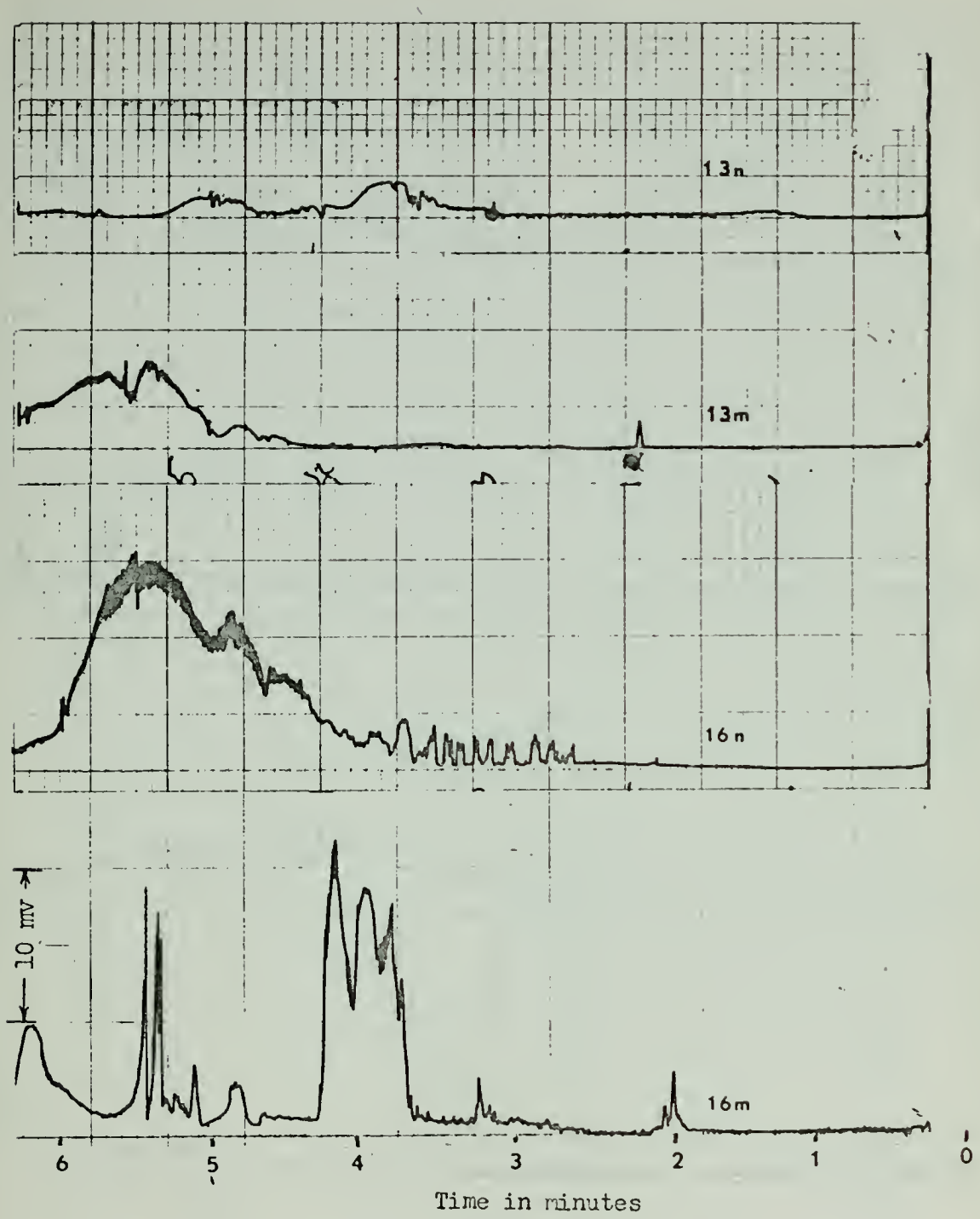


Figure 18. First four tests of laboratory euphausiid #5. No. is day of month, n = noon test, m = midnight test.

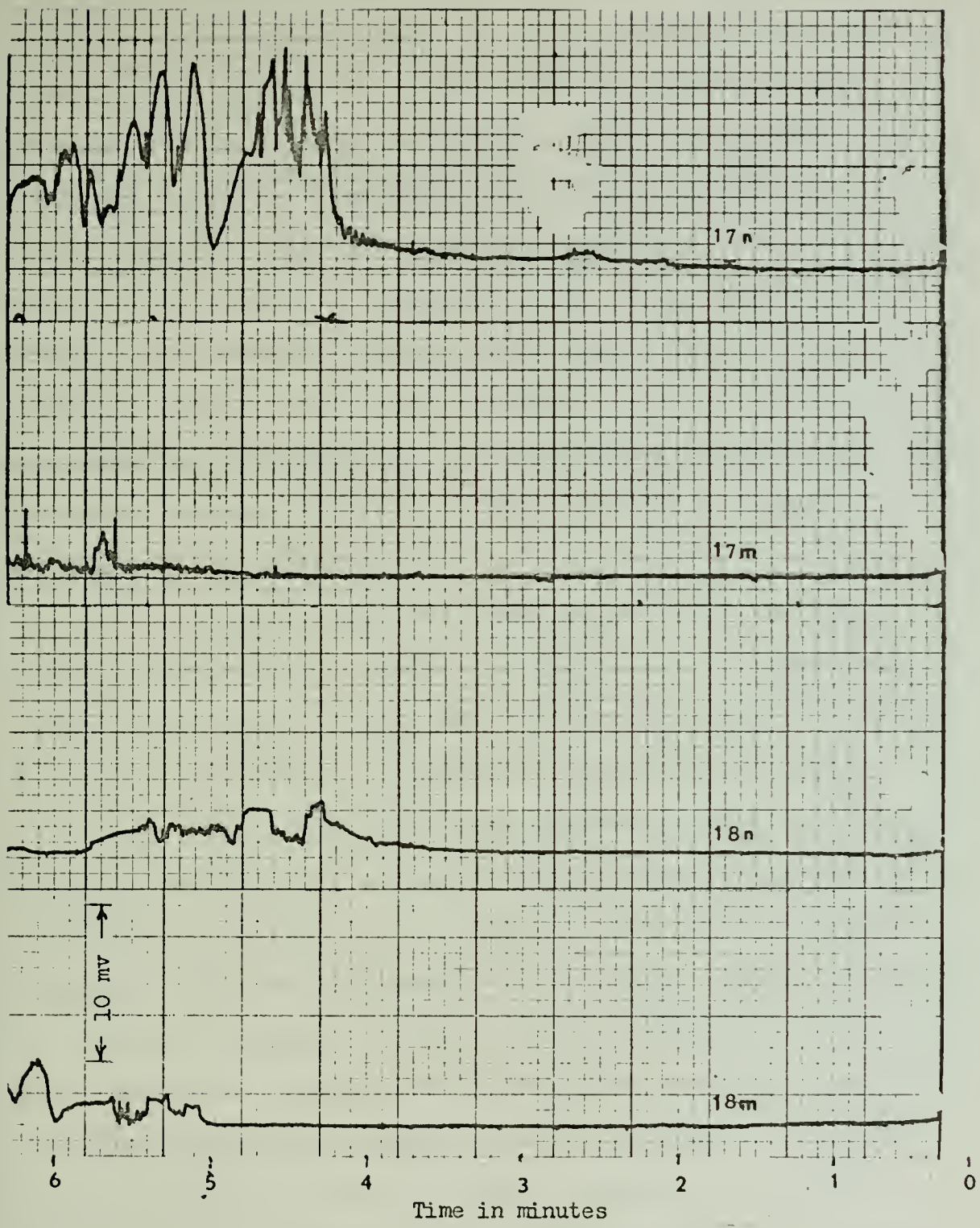


Figure 19. Second four tests of laboratory euphausiid #5. No. is day of month, n = noon test, m = midnight test.

planimeter (Dietzgen Model D-1806). This area in square centimeters was then divided by 2.5 to normalize area to the other scaled parameters. Flash rate was found and the average rate per minute calculated. Only deflections greater than 2 mm in height were counted as flashes. In cases where the flash rate was irregular the total number of flashes was counted and divided by the total elapsed time to obtain the number of flashes per minute. If the flash rate appeared to be regular, two one-minute portions of the record were counted and averaged. The maximum amplitude or deviation above the background level was measured and recorded in centimeters deflection.

Occasionally the light output would be high enough to drive the recorder pen off scale. This was not compensated for in area or amplitude measurements, as only a relative indication of euphausiid activity was sought.

The activity of each euphausiid was then charted for each parameter at each test period from the results of the above evaluations. The date and time of test are along the upper abscissa (Figures 20-40). A noon test is indicated by "n" and a midnight test by an "m". Moulting is indicated along the lower abscissa by an "M". The measured parameters are listed along the right ordinate. A break in the graph indicates that more than 24 hours have passed since the previous test. Symbols at the top of the record indicate that the measurement of the parameter in question has gone off scale.

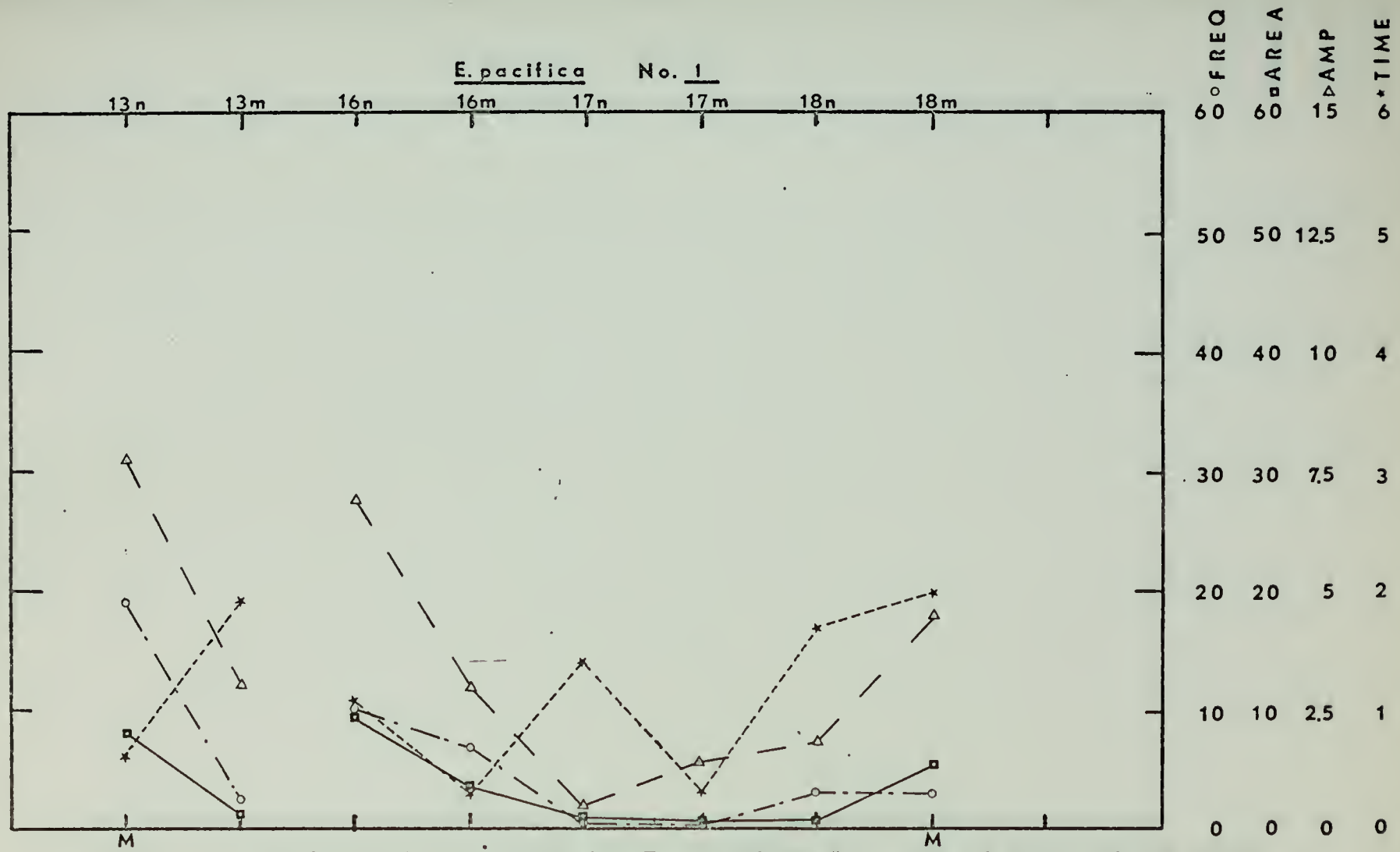


Figure 20. Daily activity chart for E. pacifica #1. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

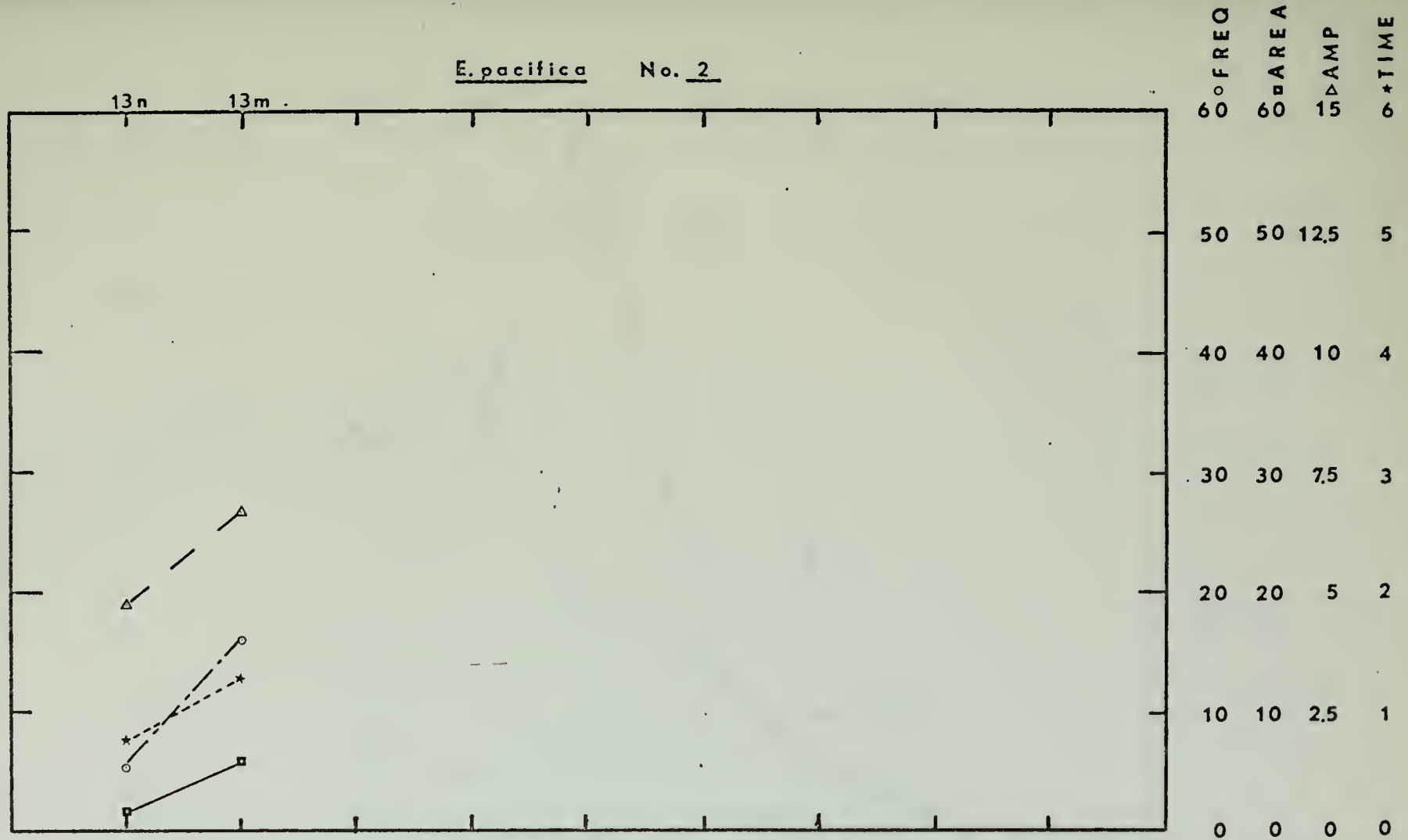


Figure 21. Daily activity chart for E. pacifica #2. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

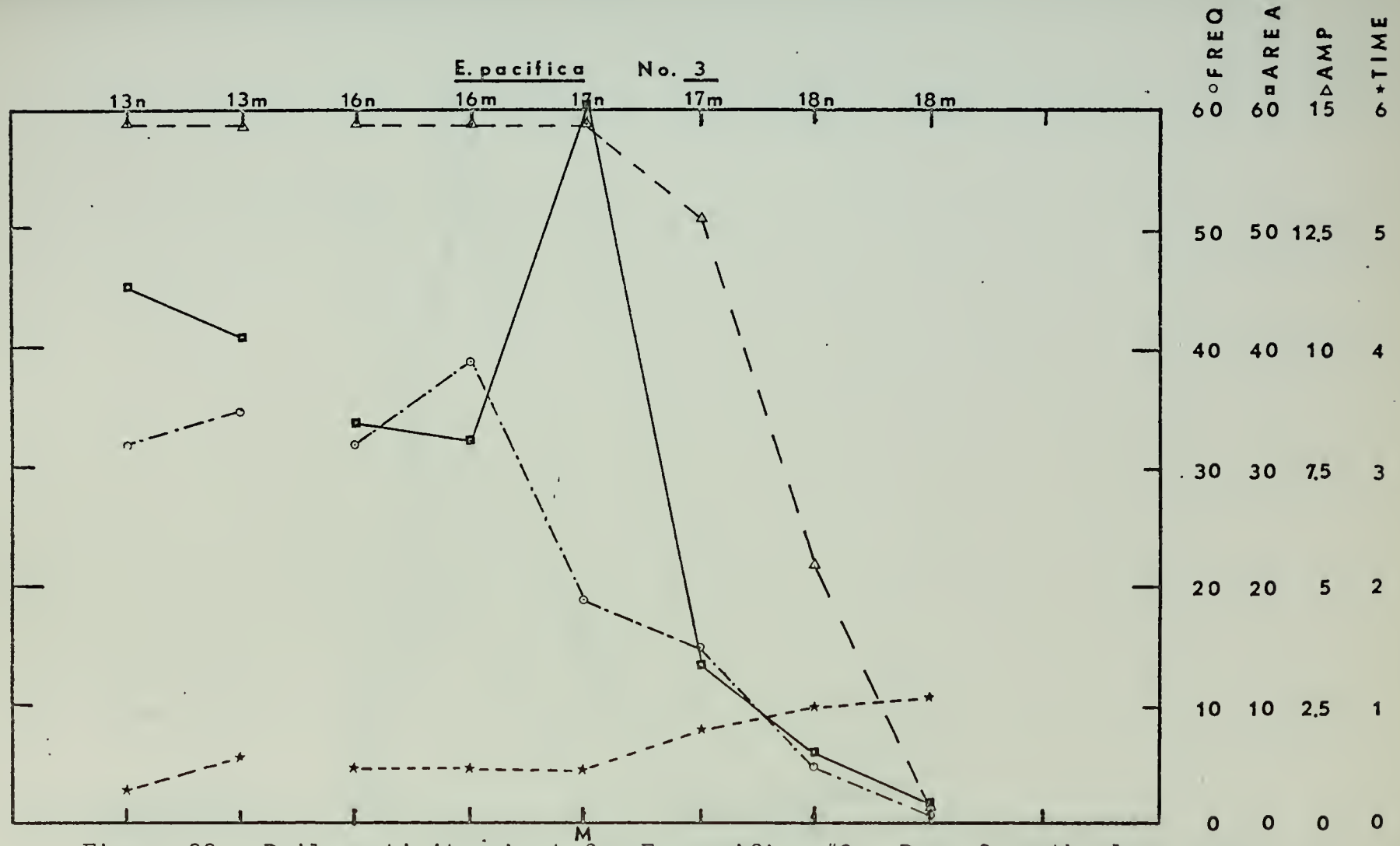


Figure 22. Daily activity chart for E. pacifica #3. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

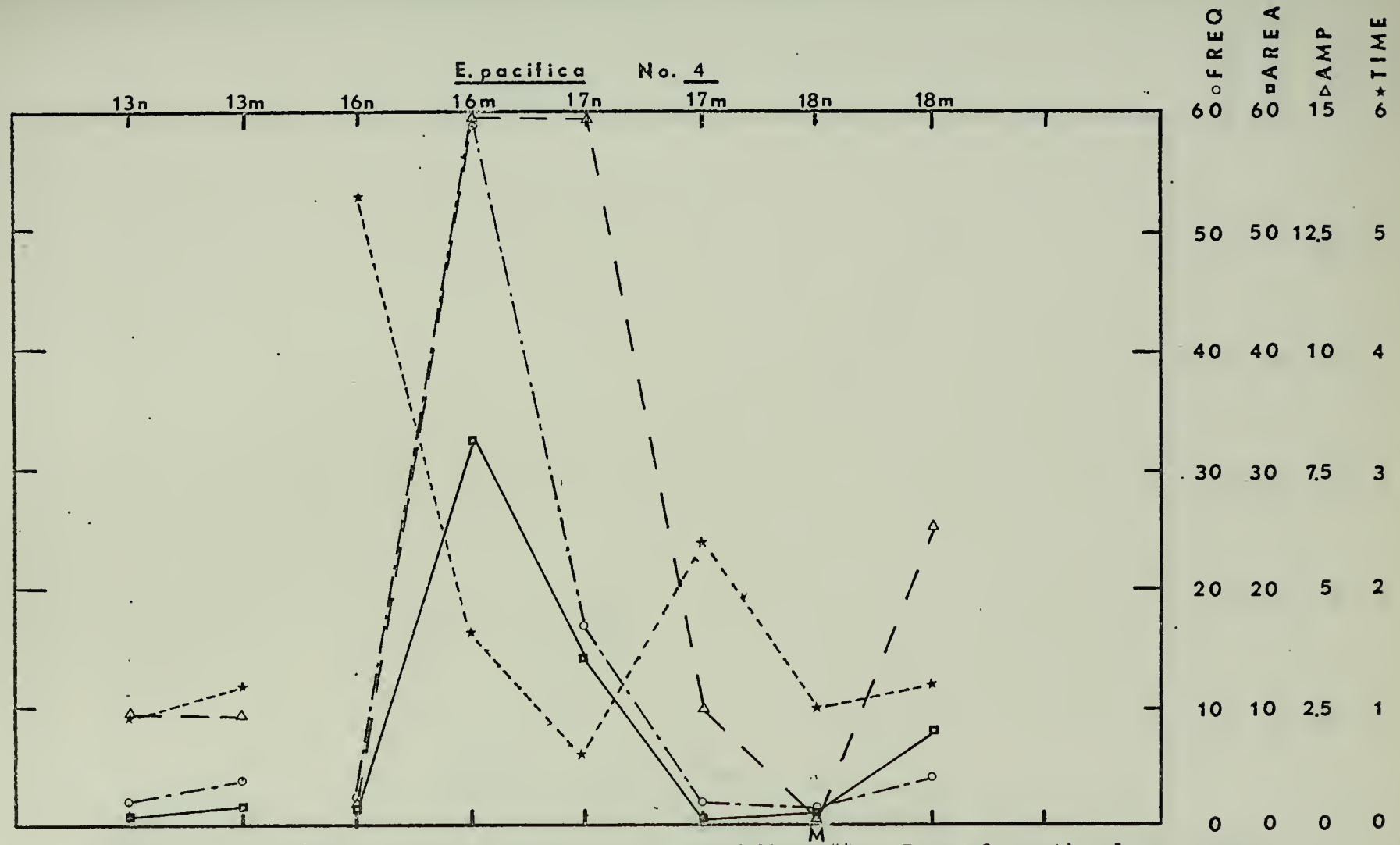


Figure 23. Daily activity chart for E. pacifica #4. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

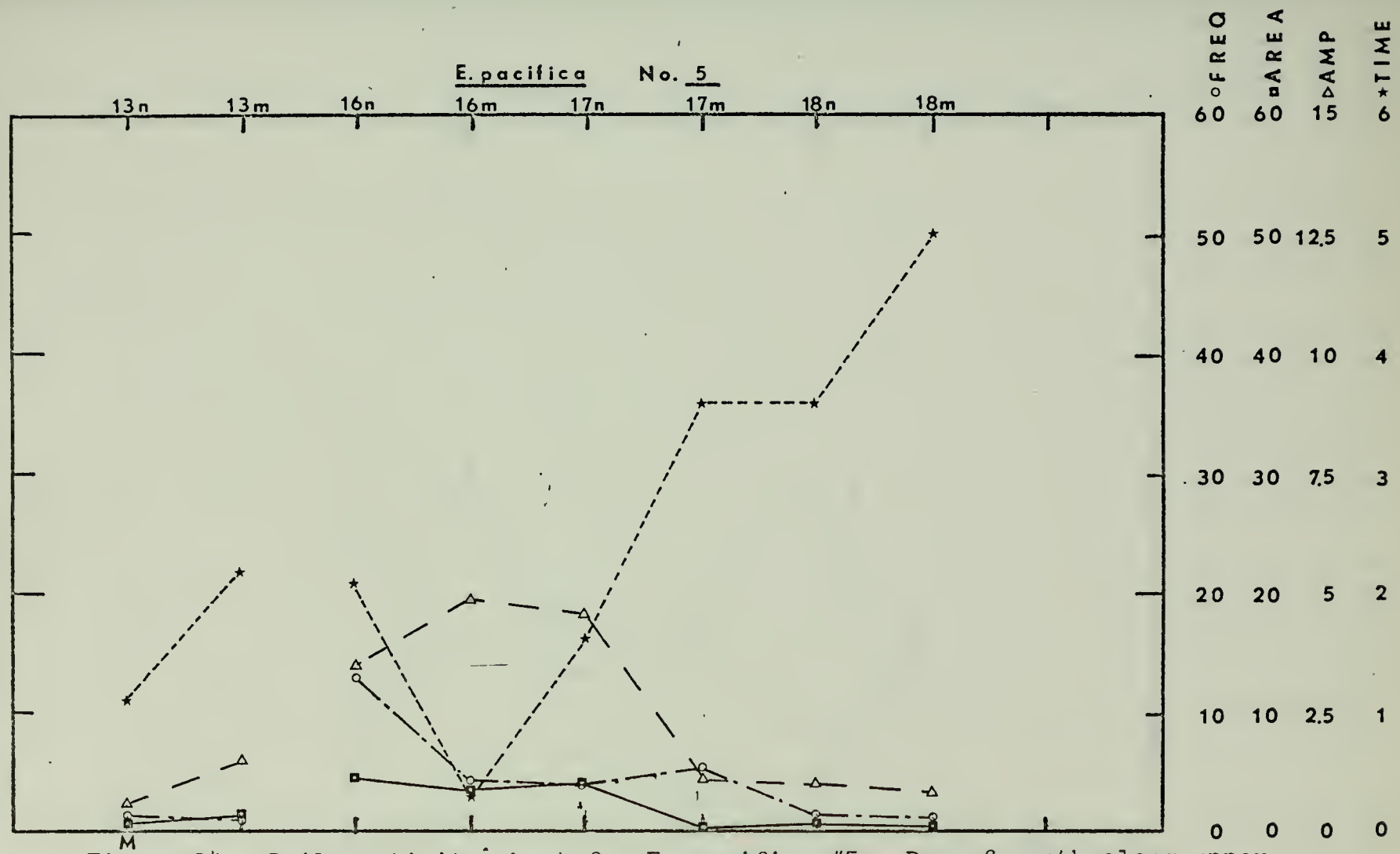


Figure 24. Daily activity chart for E. pacifica #5. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

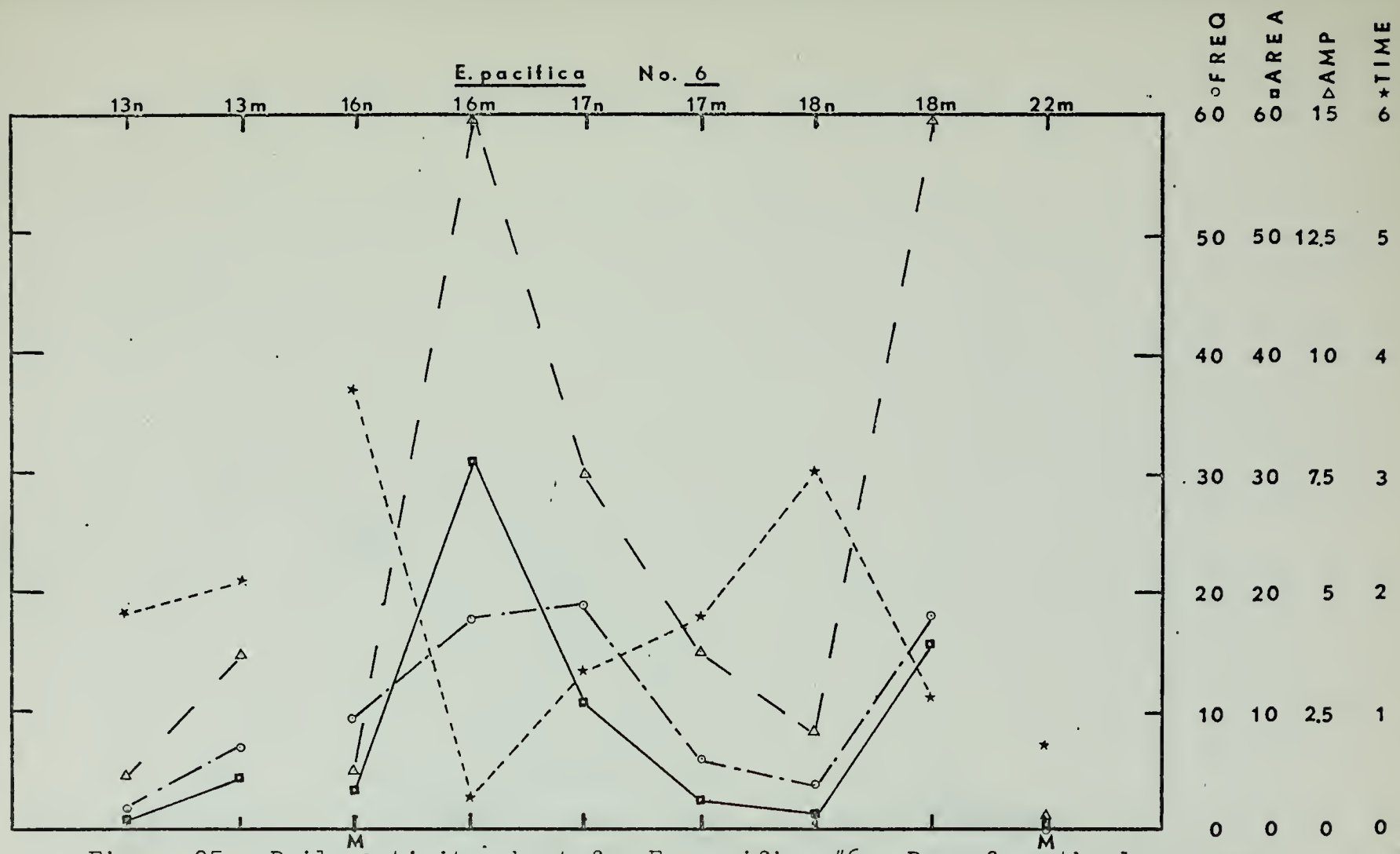


Figure 25. Daily activity chart for E. pacifica #6. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

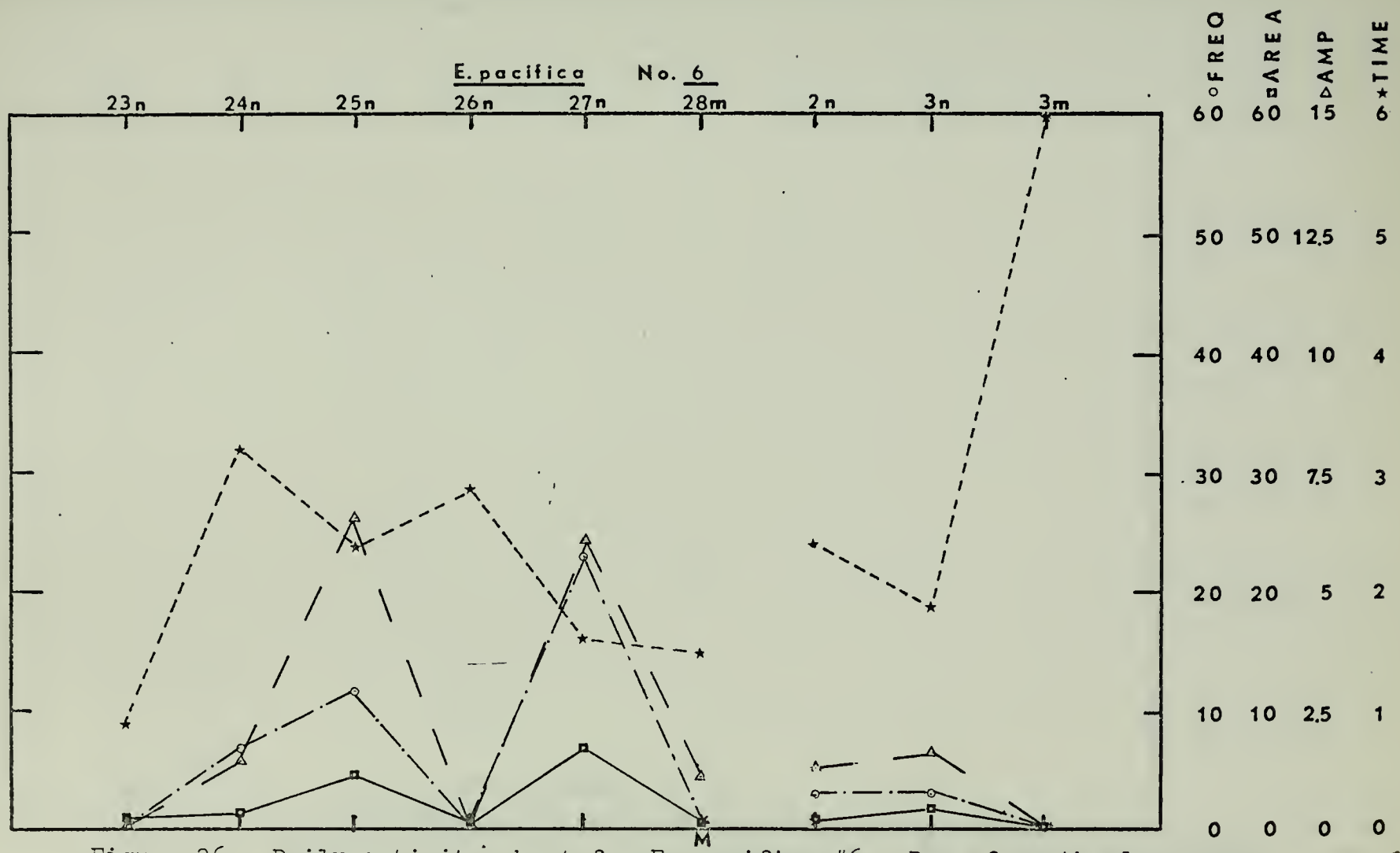


Figure 26. Daily activity chart for E. pacifica #6. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

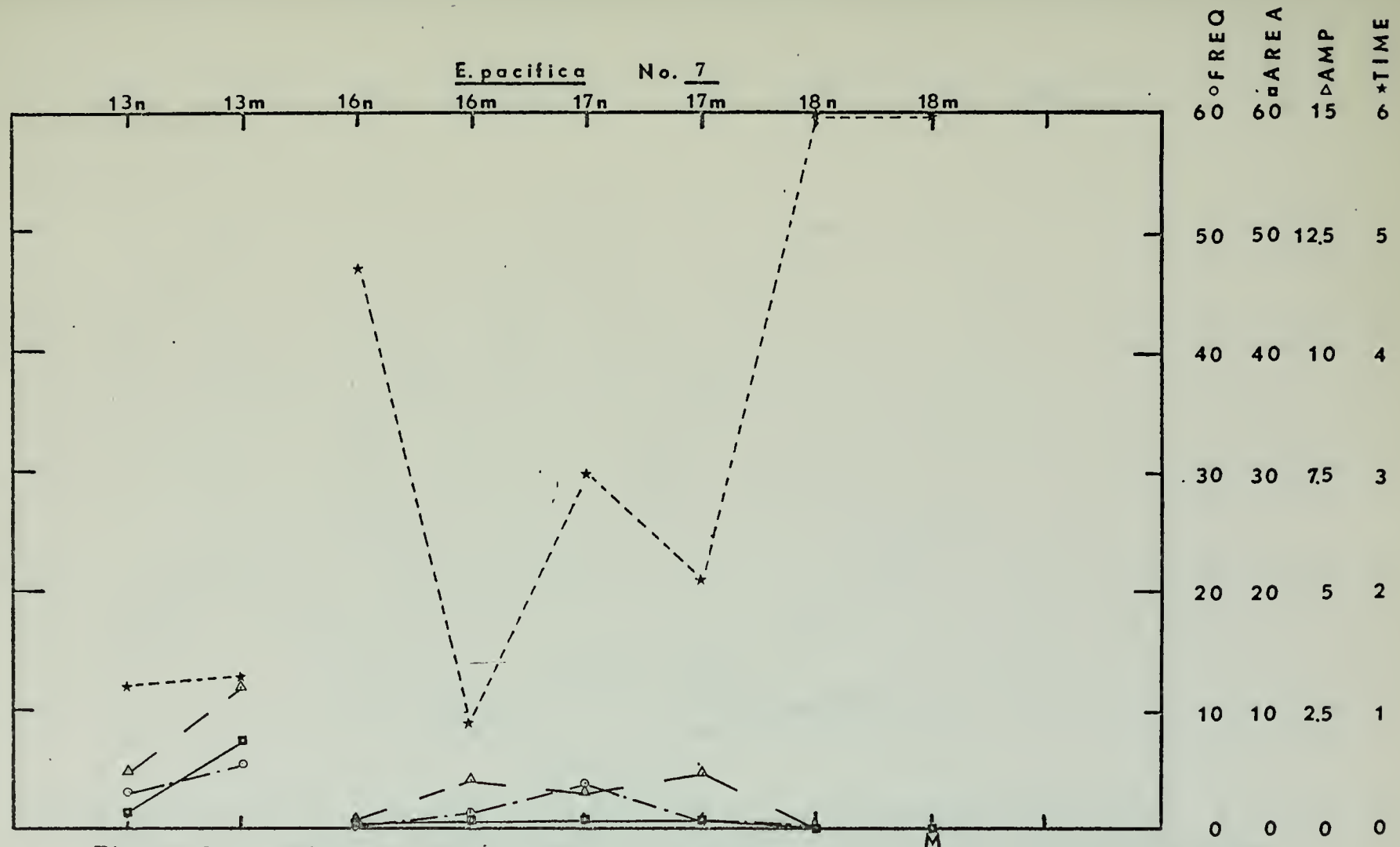


Figure 27. Daily activity chart for E. pacifica #7. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

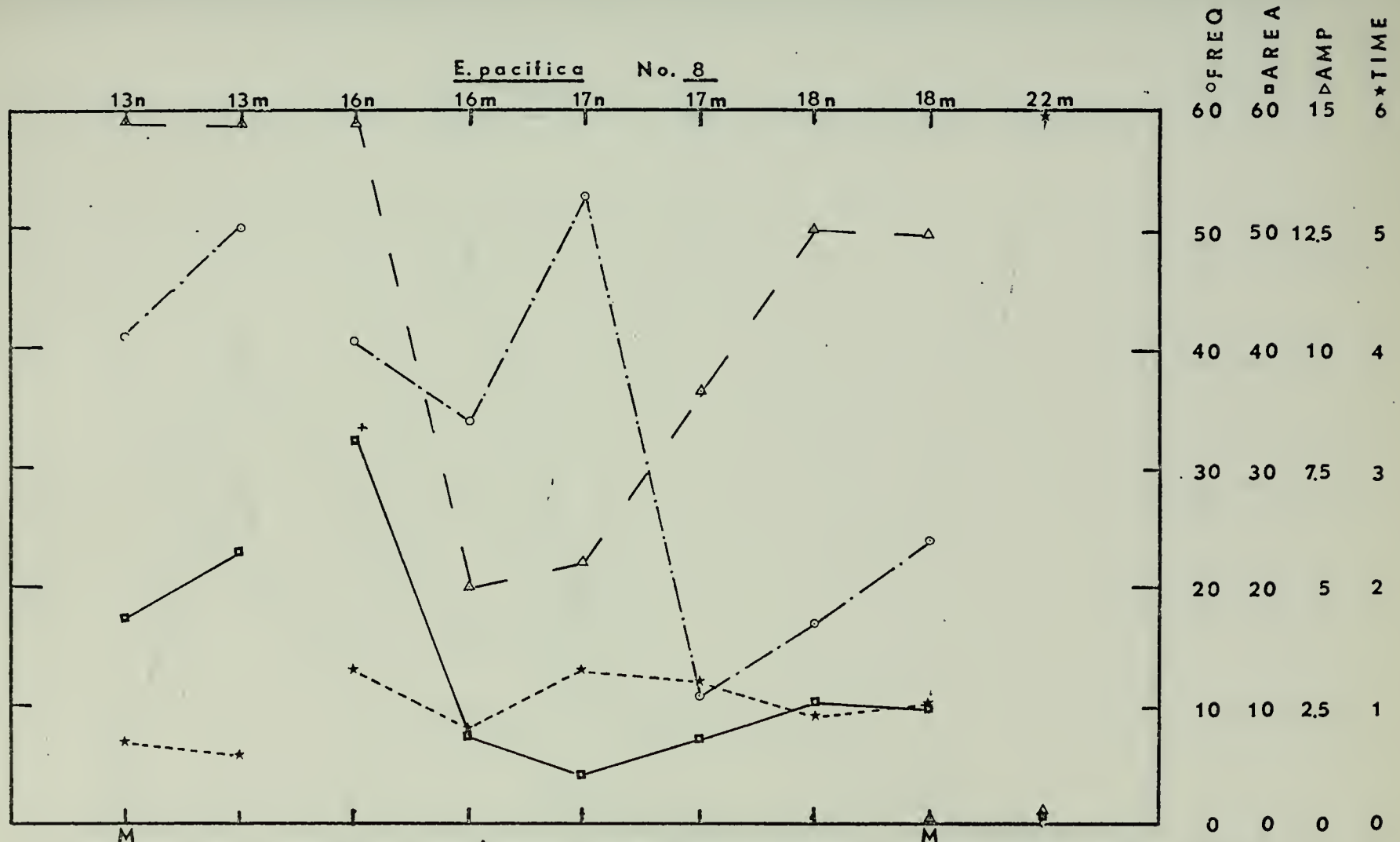


Figure 28. Daily activity chart for E. pacifica #8. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

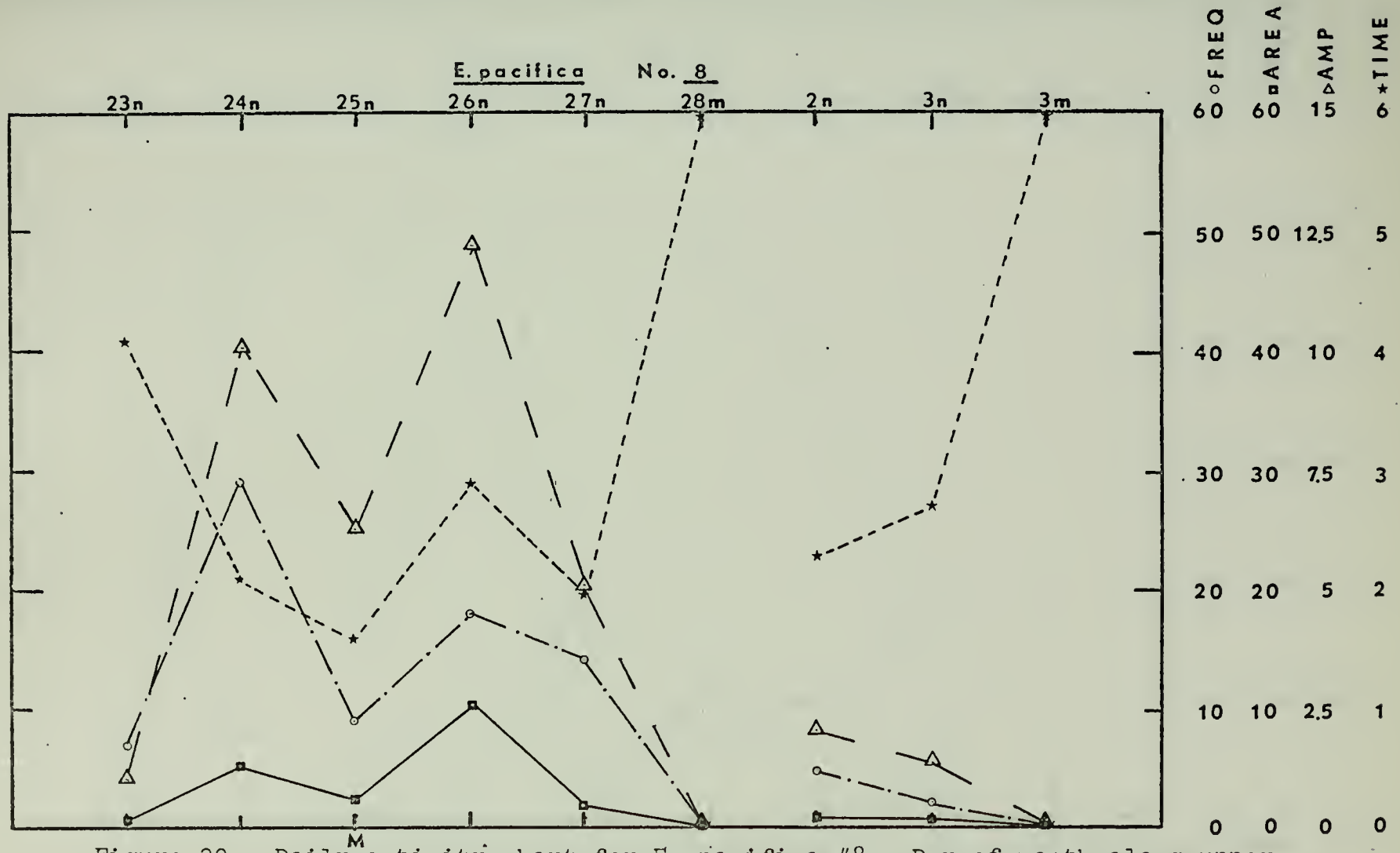


Figure 29. Daily activity chart for E. pacifica #8. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

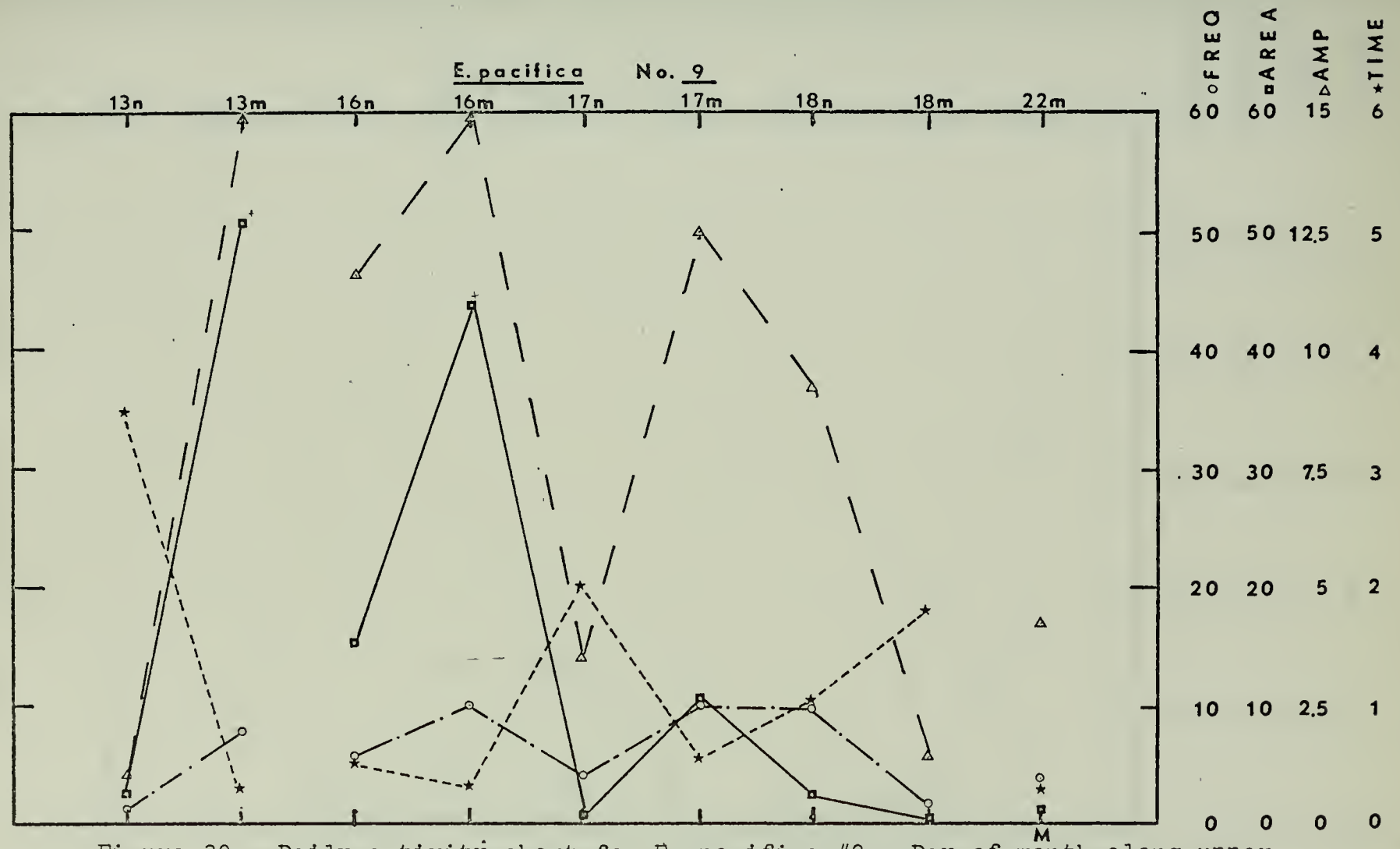


Figure 30. Daily activity chart for E. pacifica #9. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

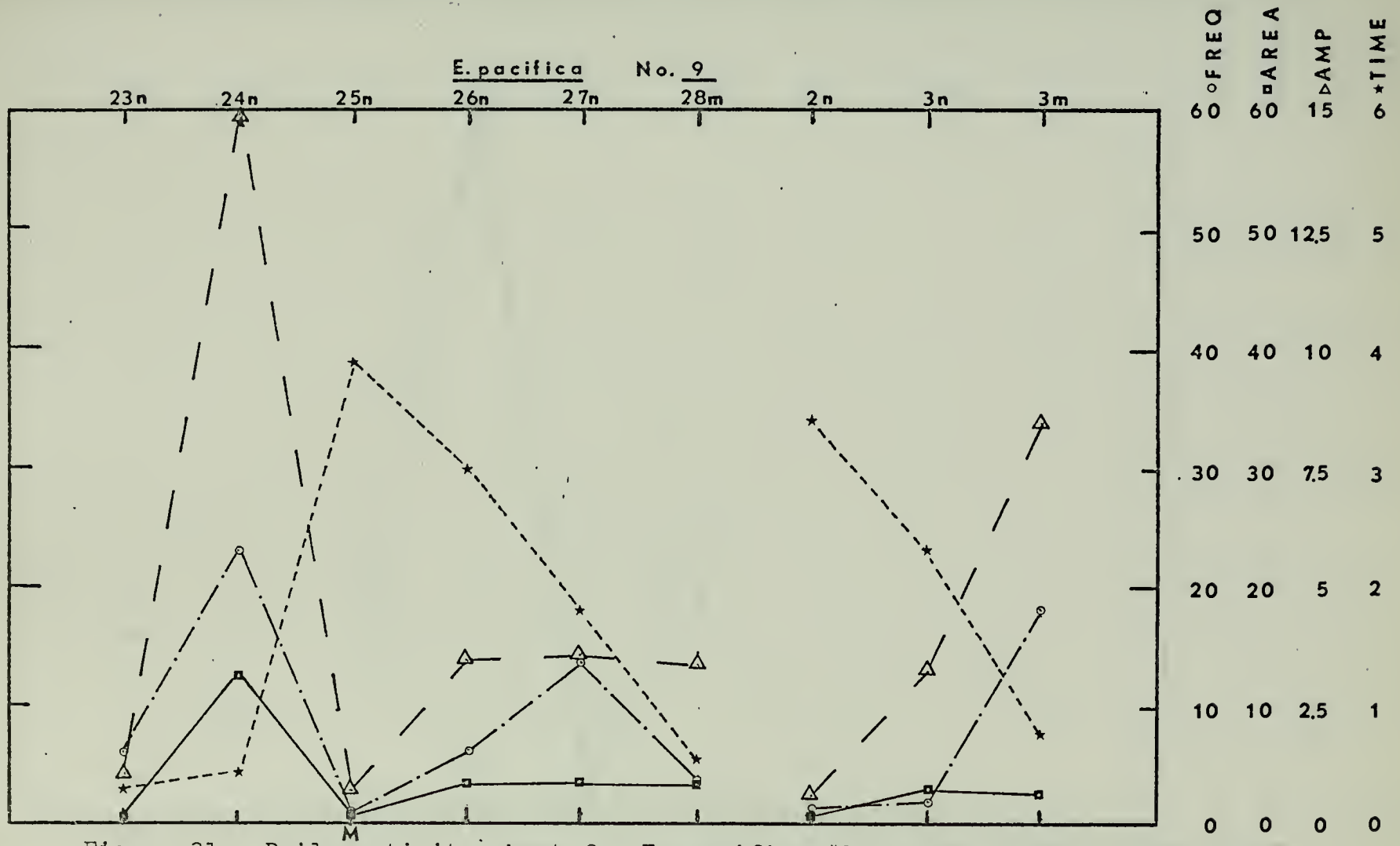


Figure 31. Daily activity chart for E. pacifica #9. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

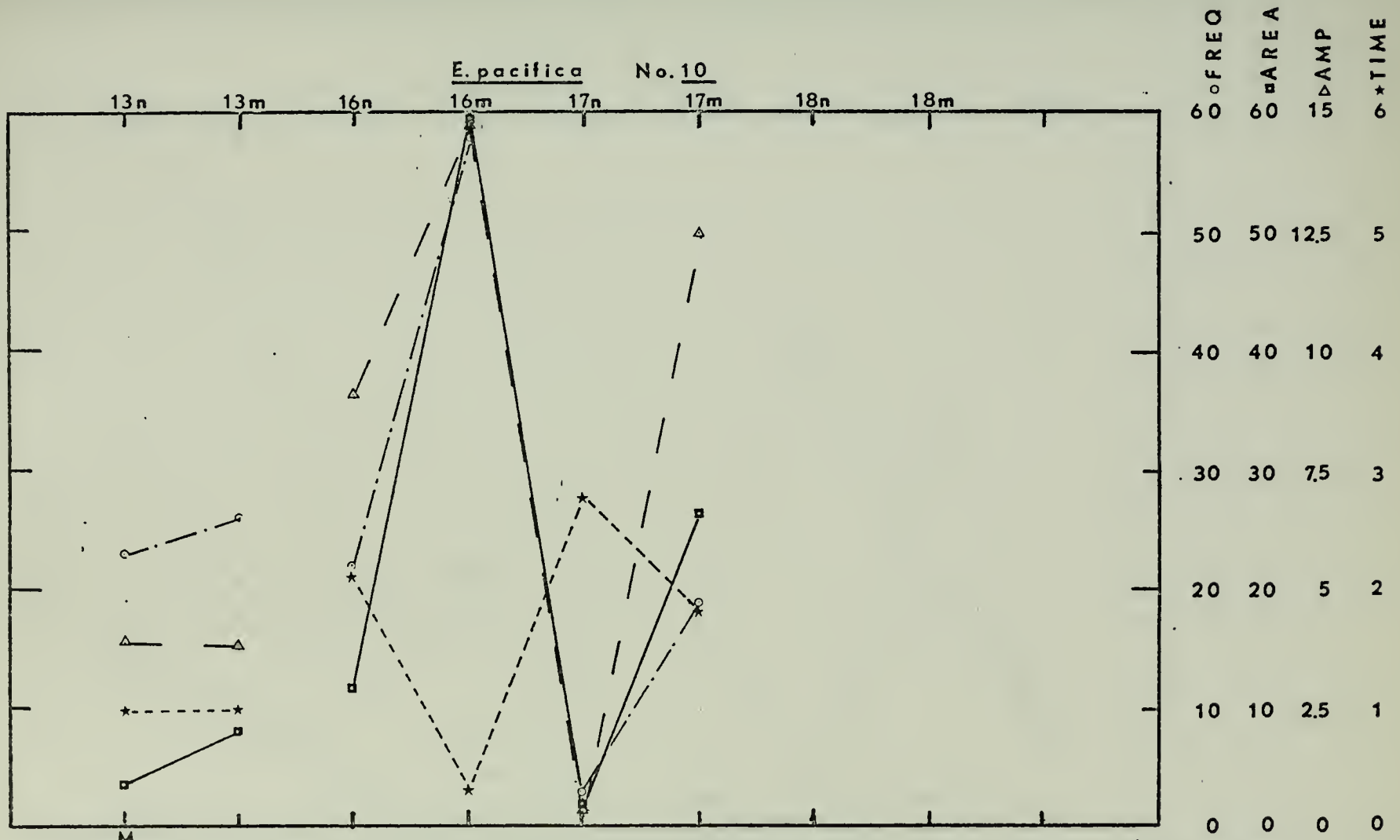


Figure 32. Daily activity chart for E. pacifica #10. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

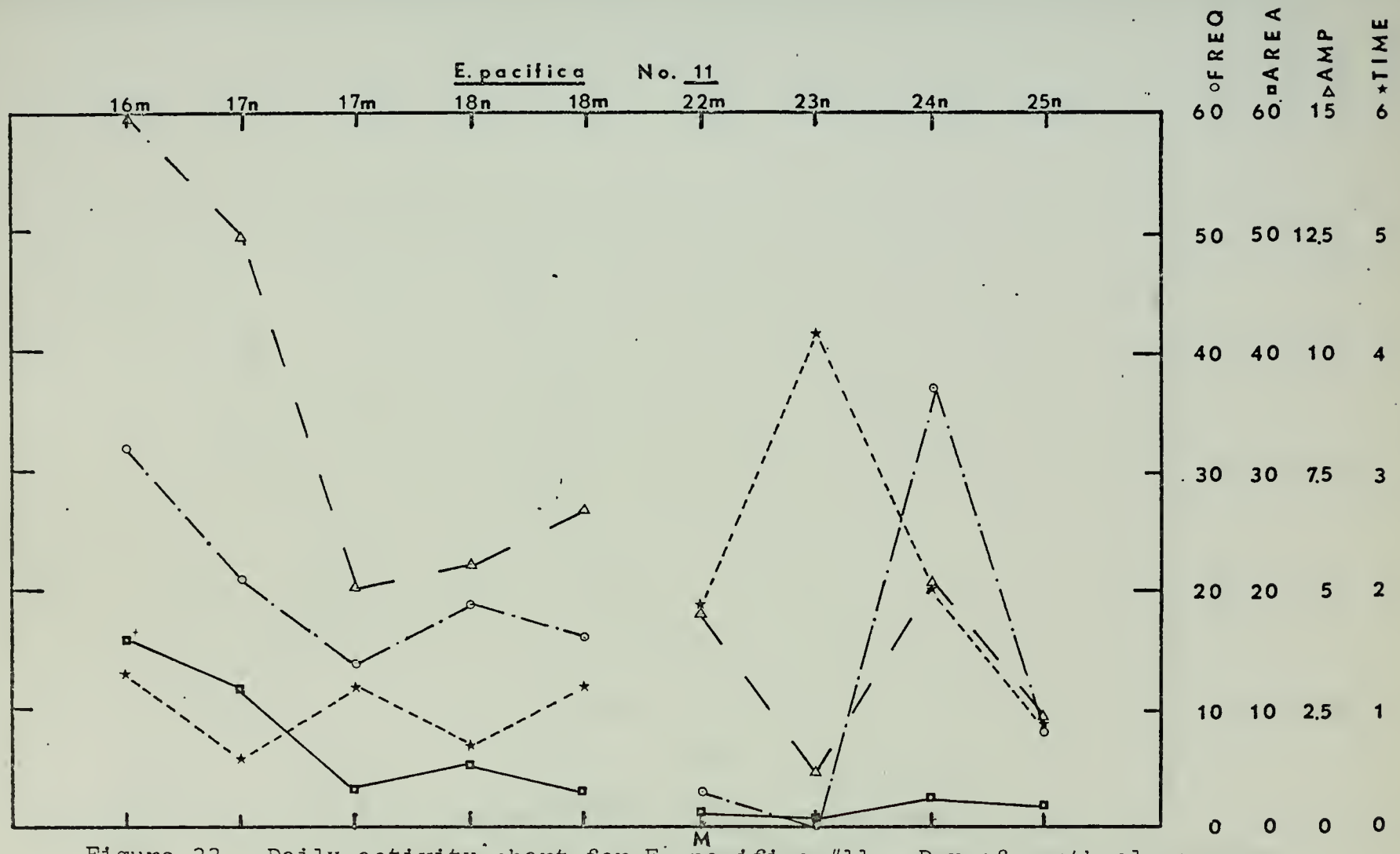


Figure 33. Daily activity chart for E. pacifica #11. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

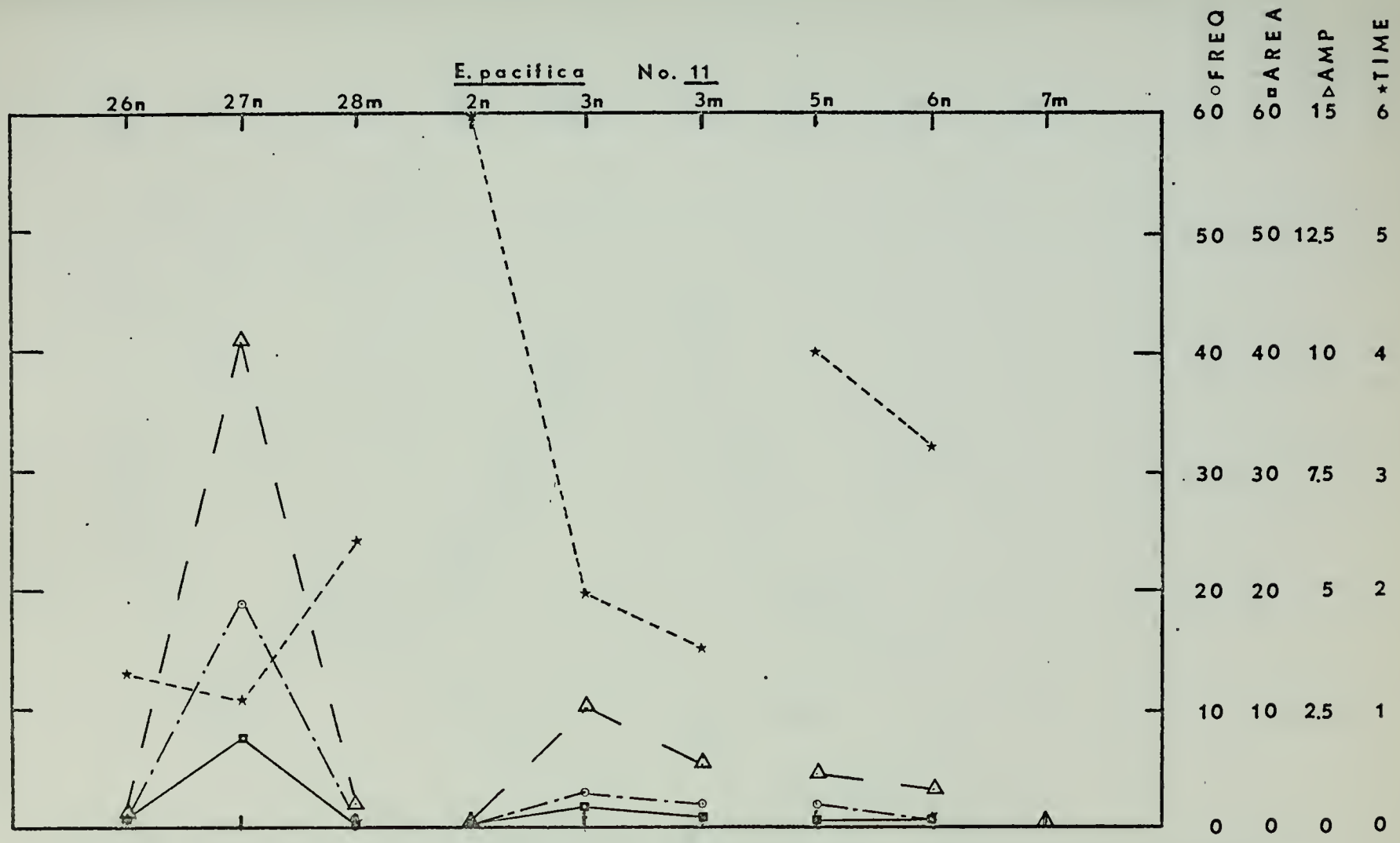


Figure 34. Daily activity chart for E. pacifica #11. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

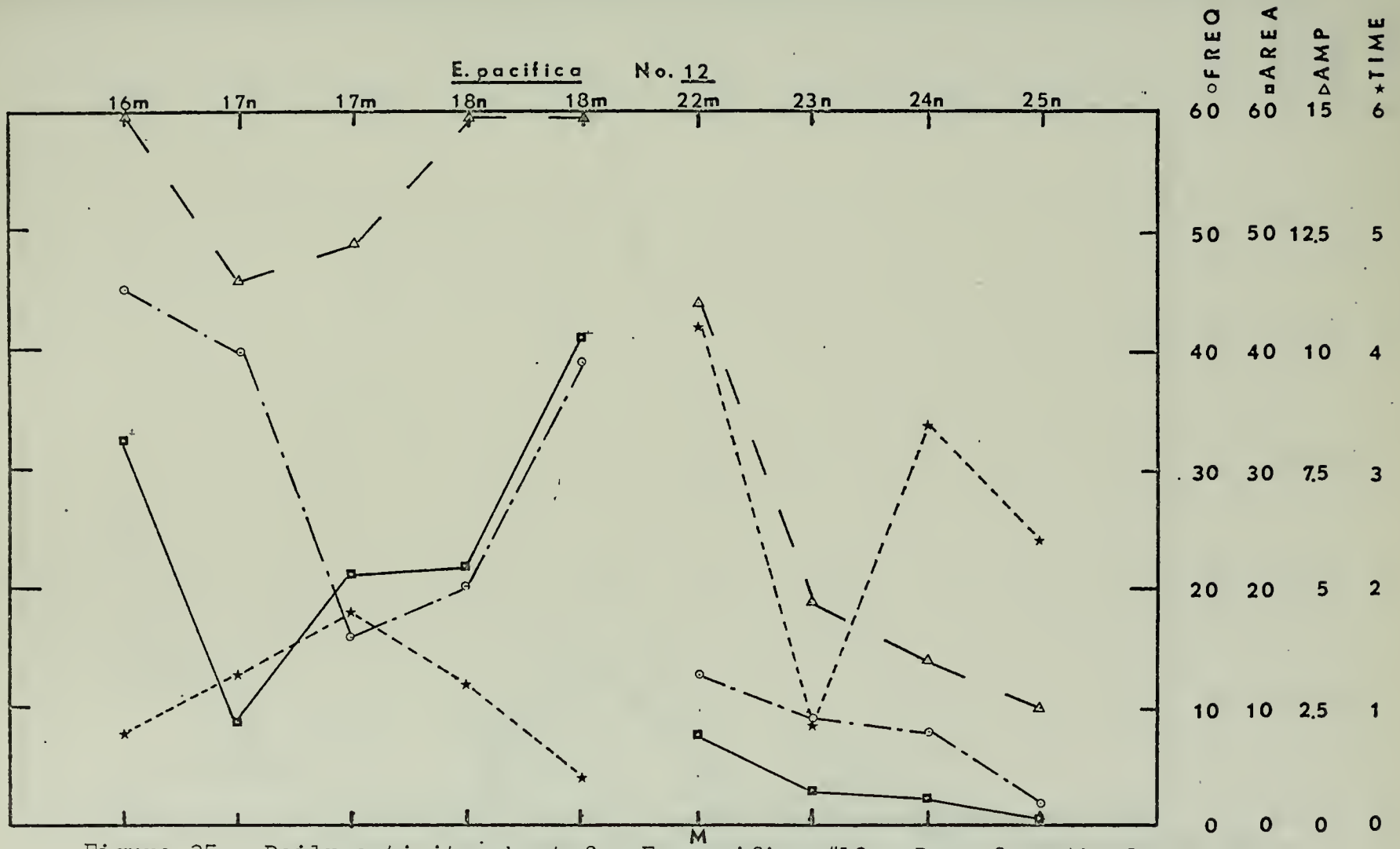


Figure 35. Daily activity chart for E. pacifica #12. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

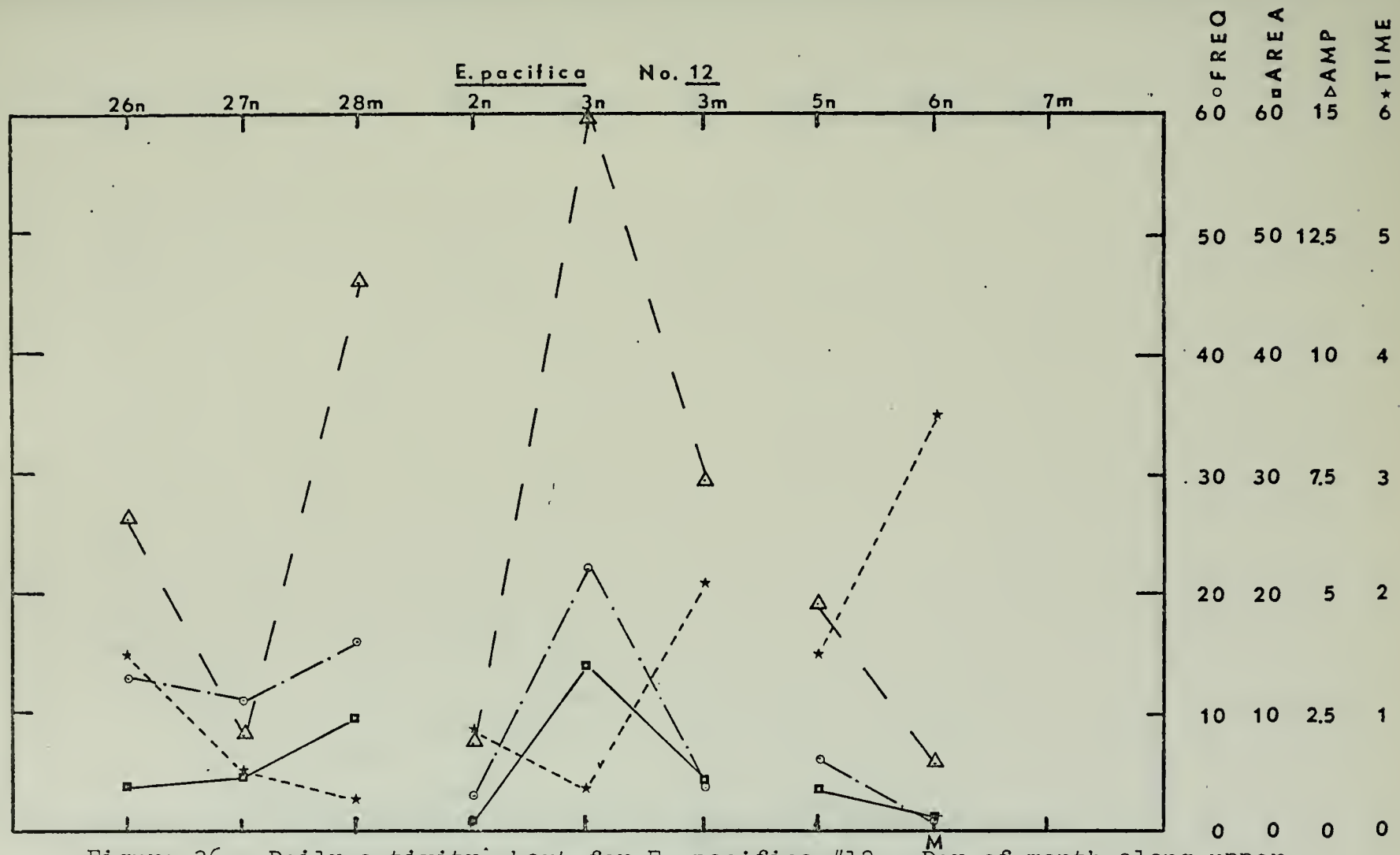


Figure 36. Daily activity chart for E. pacifica #12. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

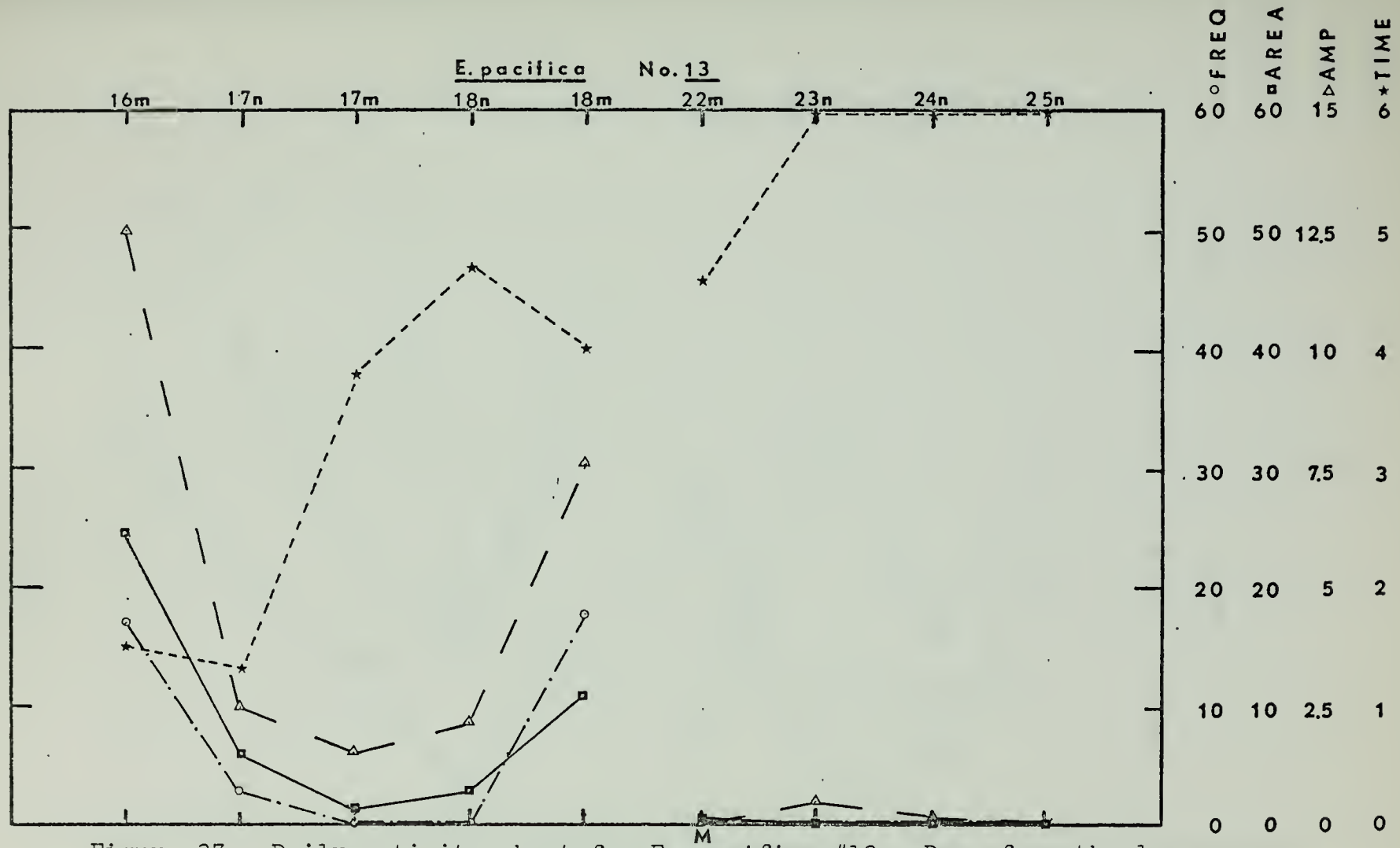


Figure 37. Daily activity chart for E. pacifica #13. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

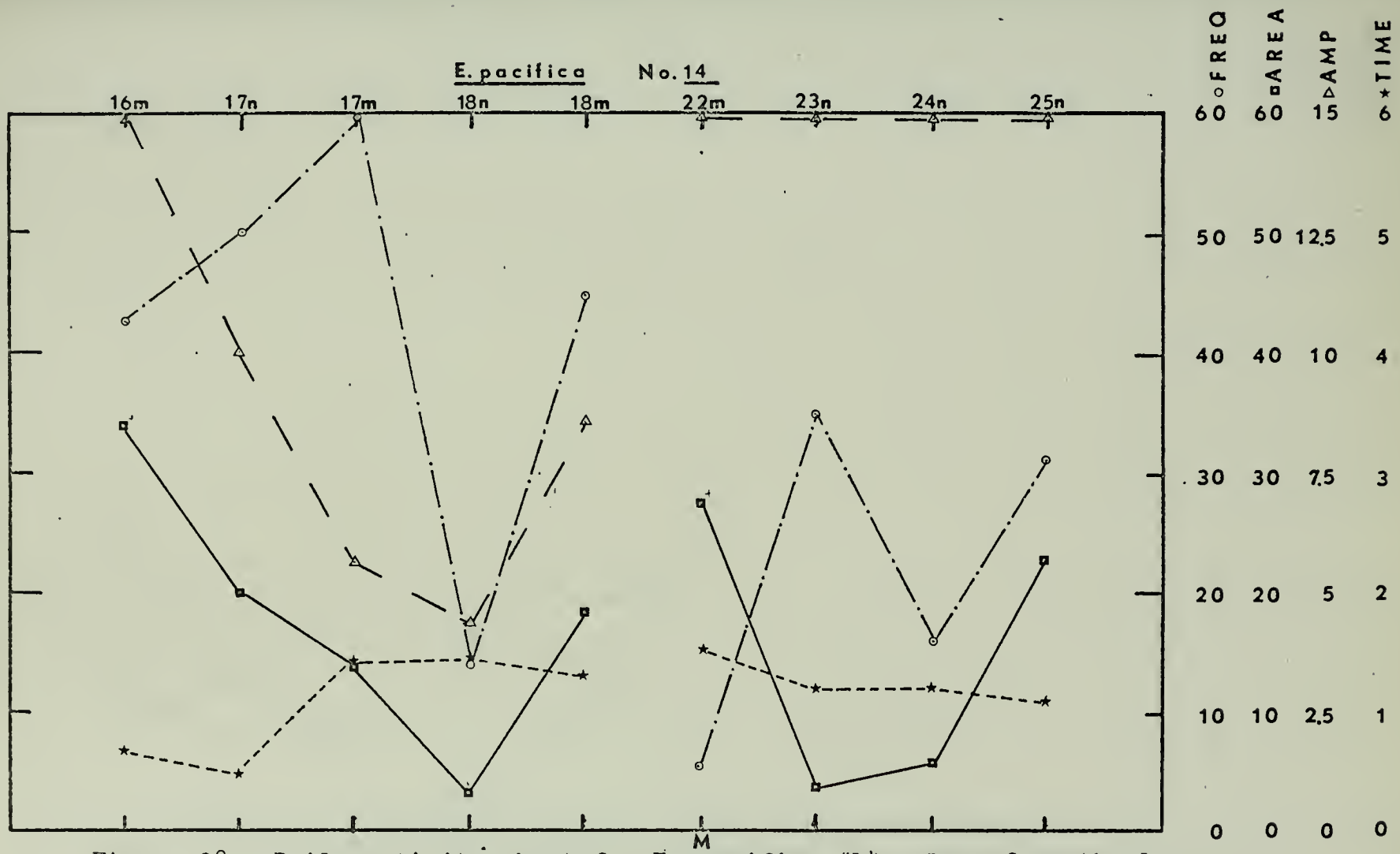


Figure 38. Daily activity chart for E. pacifica #14. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

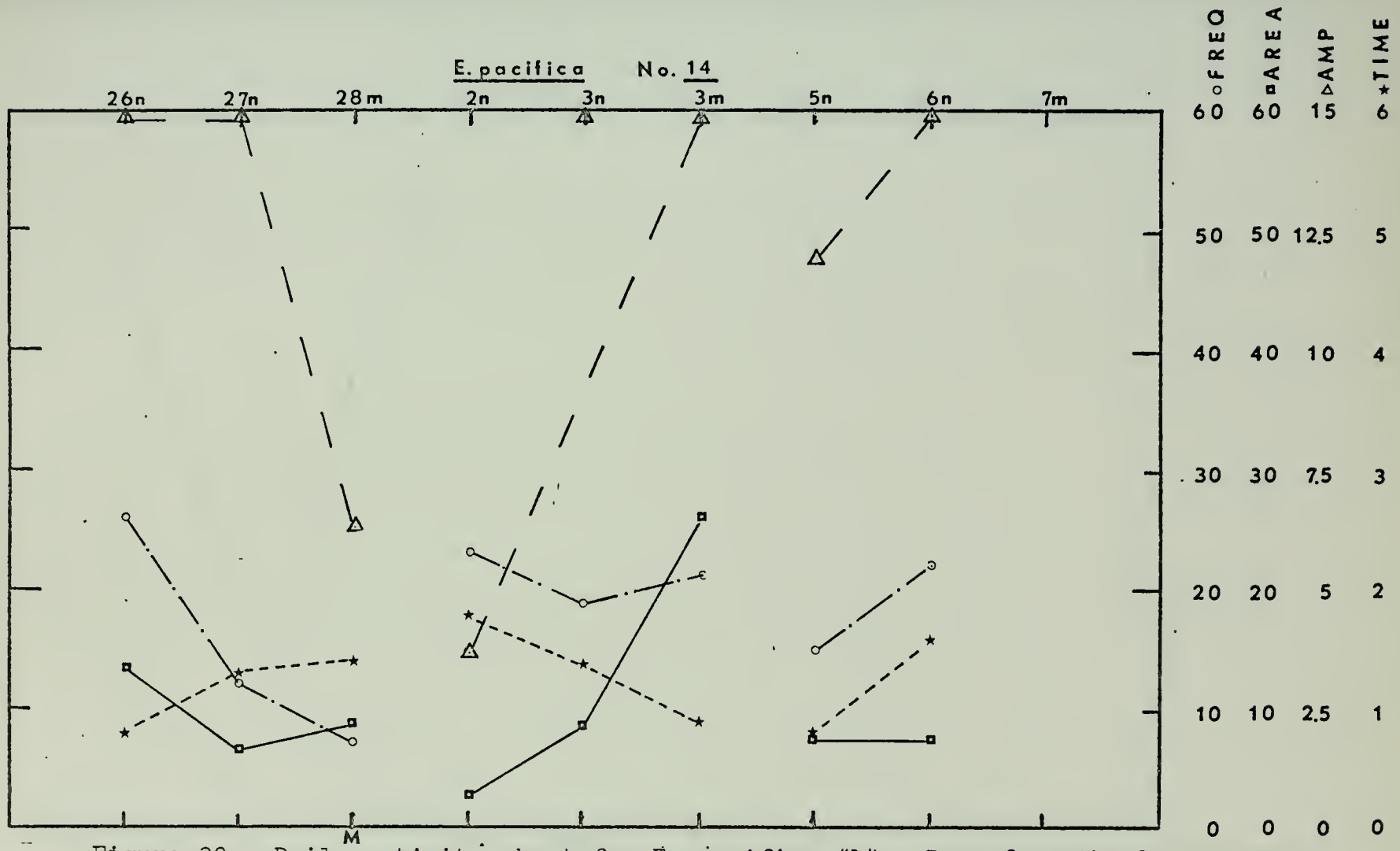


Figure 39. Daily activity chart for E. pacifica #14. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

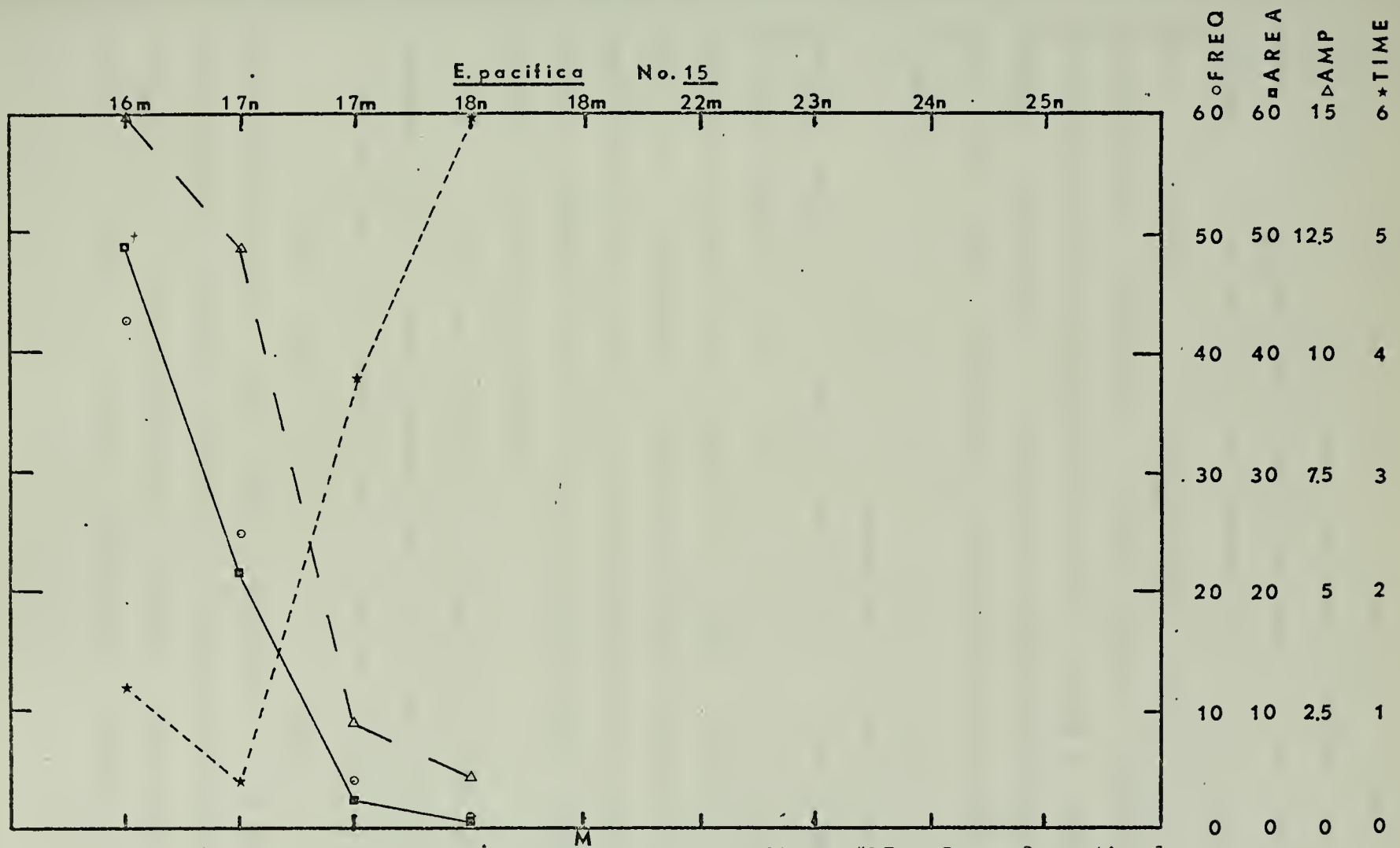


Figure 40. Daily activity chart for E. pacifica #15. Day of month along upper abscissa. "n" implies noon test. "m" implies midnight test. "M" along lower abscissa implies moult formed. Flash rate in flashes per minute. Light output as area in $cm^2/2.5$. Maximum amplitude in cm. Reaction time in minutes.

Tests were stopped for a particular organism if it died, if no activity was displayed for three consecutive tests, or if the euphausiid would not swim up into the test chamber. At the end of 23 days seven of fifteen euphausiids were still alive, but only one was still bioluminescent, so the tests were terminated. (A control group, to determine deterioration of bioluminescent activity caused by daily testing, was not kept.) Five of the eight euphausiids that died did so after a temperature control failure in the laboratory. All of test group one were lost at this time.

The results of the parameter evaluations were further analyzed by averaging the activity of each parameter for each euphausiid for: total activity, noon activity, midnight activity and a "before" and "after" moulting (Table II). The moults, found at test time could not be associated with a specific test; therefore the test just prior to finding the moult and the test just after finding the moult were both evaluated.

After the individual averages were made, group averages (Table 3) were accomplished to evaluate the effect of temperature. The results are plotted on Figures 41-50.

These graphs indicate that two thirds of the euphausiids are more active during the middle of the night than during the middle of the day in responding to artificial stimulation. In the comparison of midnight versus noon activity: eight out of fifteen euphausiids displayed more light output at midnight than at noon; ten of fifteen greater

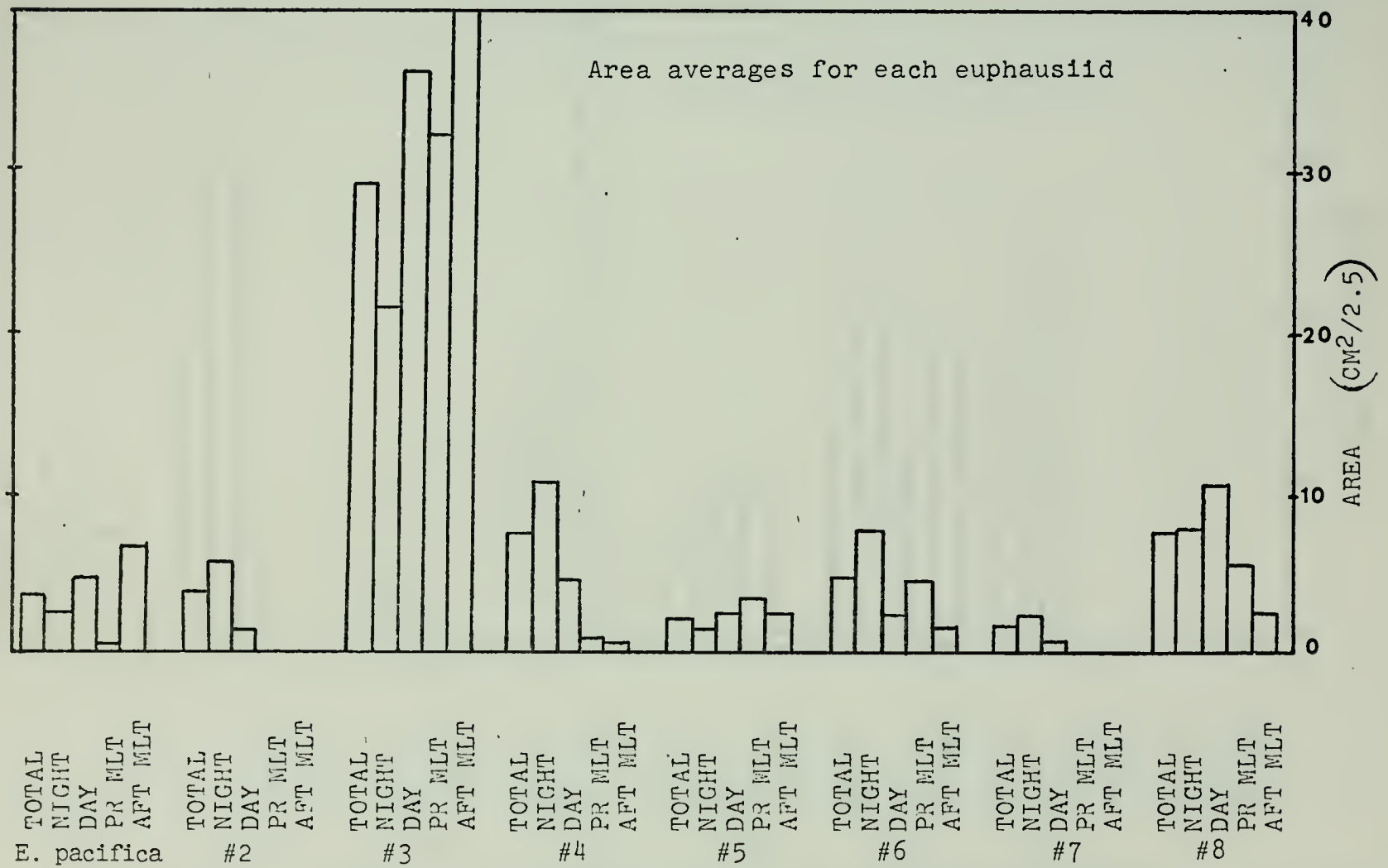


Figure 41. Individual euphausiid average for parameters indicated (PR MLT implies "prior to moulting". AFT MLT implies "after moulting").

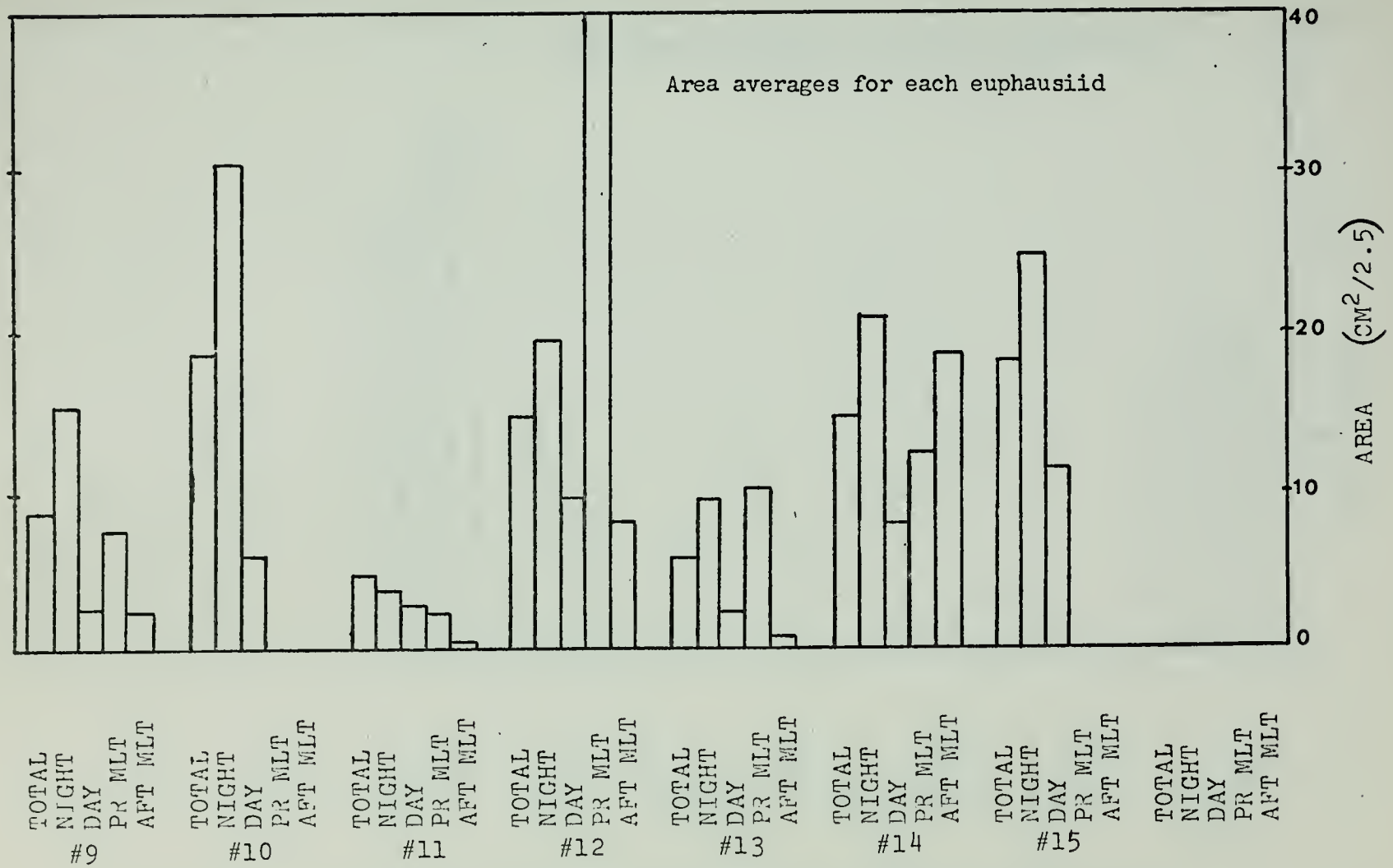


Figure 42. Individual euphausiid average for parameters indicated (PR MLT implies "prior to moulting". AFT MLT implies "after moulting").

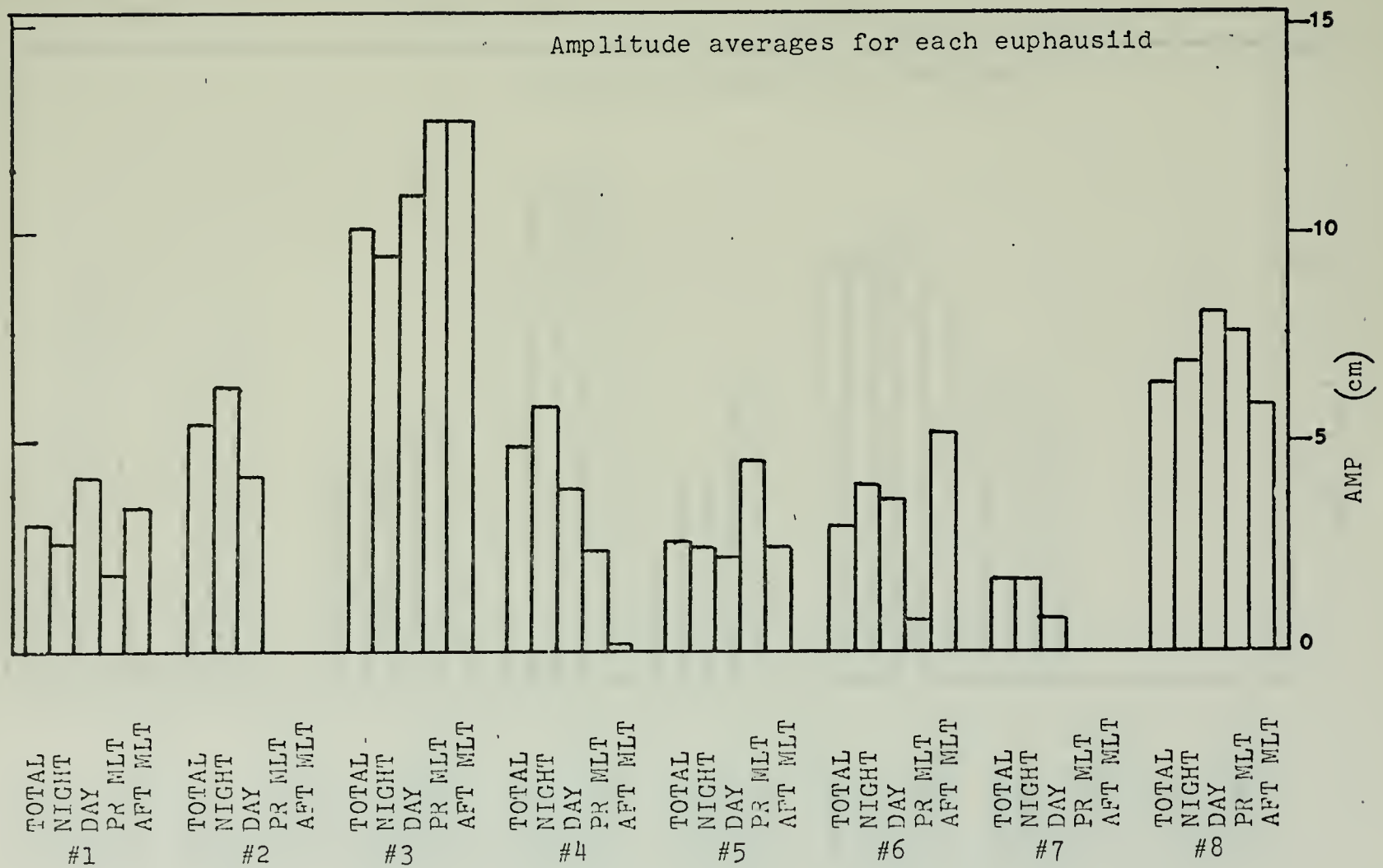


Figure 43. Individual euphausiid average for parameters indicated (PR MLT implies "prior to moulting". AFT MLT implies "after moulting").

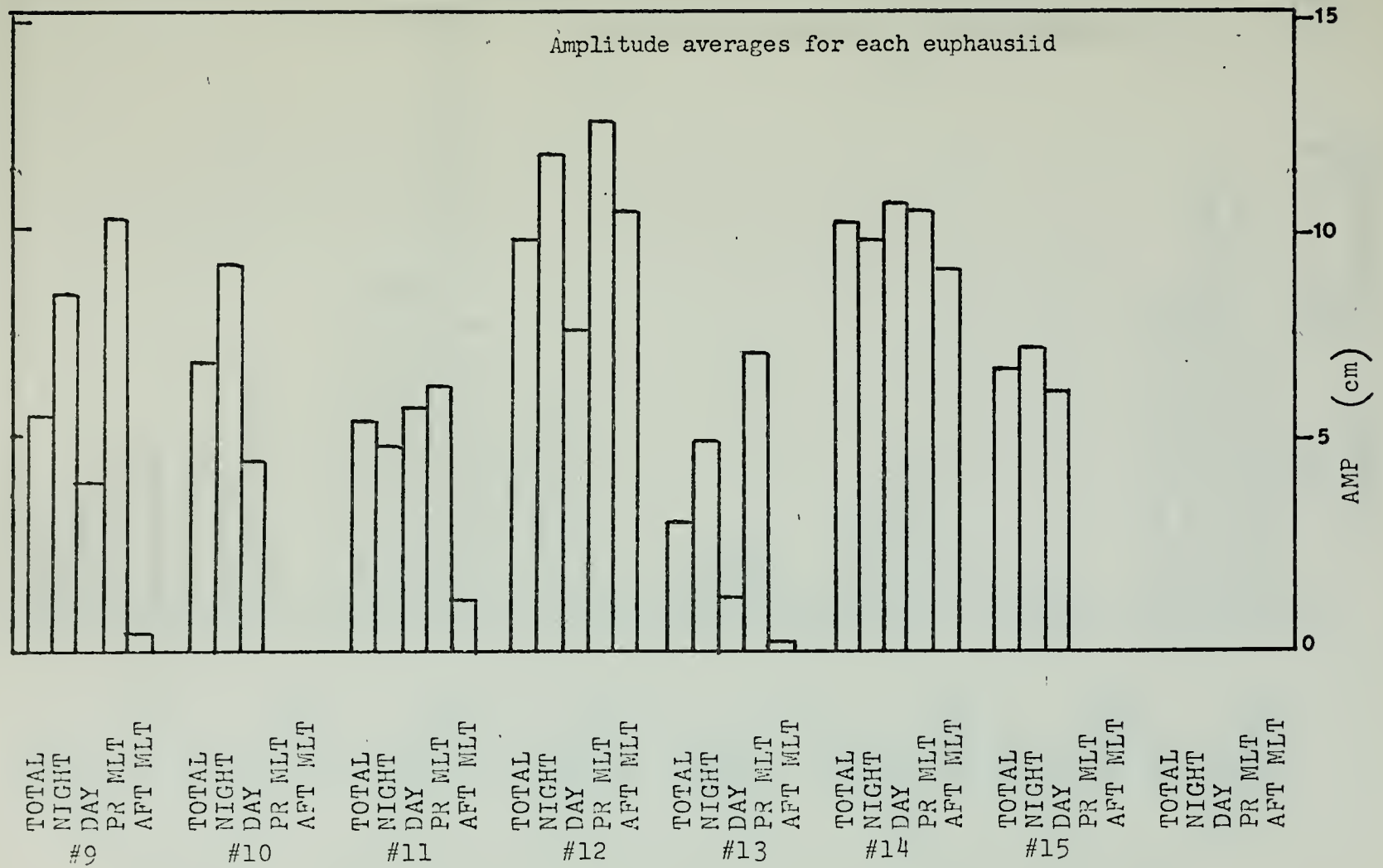


Figure 44. Individual euphausiid average for parameters indicated (PR MLT implies "prior to moulting". AFT MLT implies "after moulting").

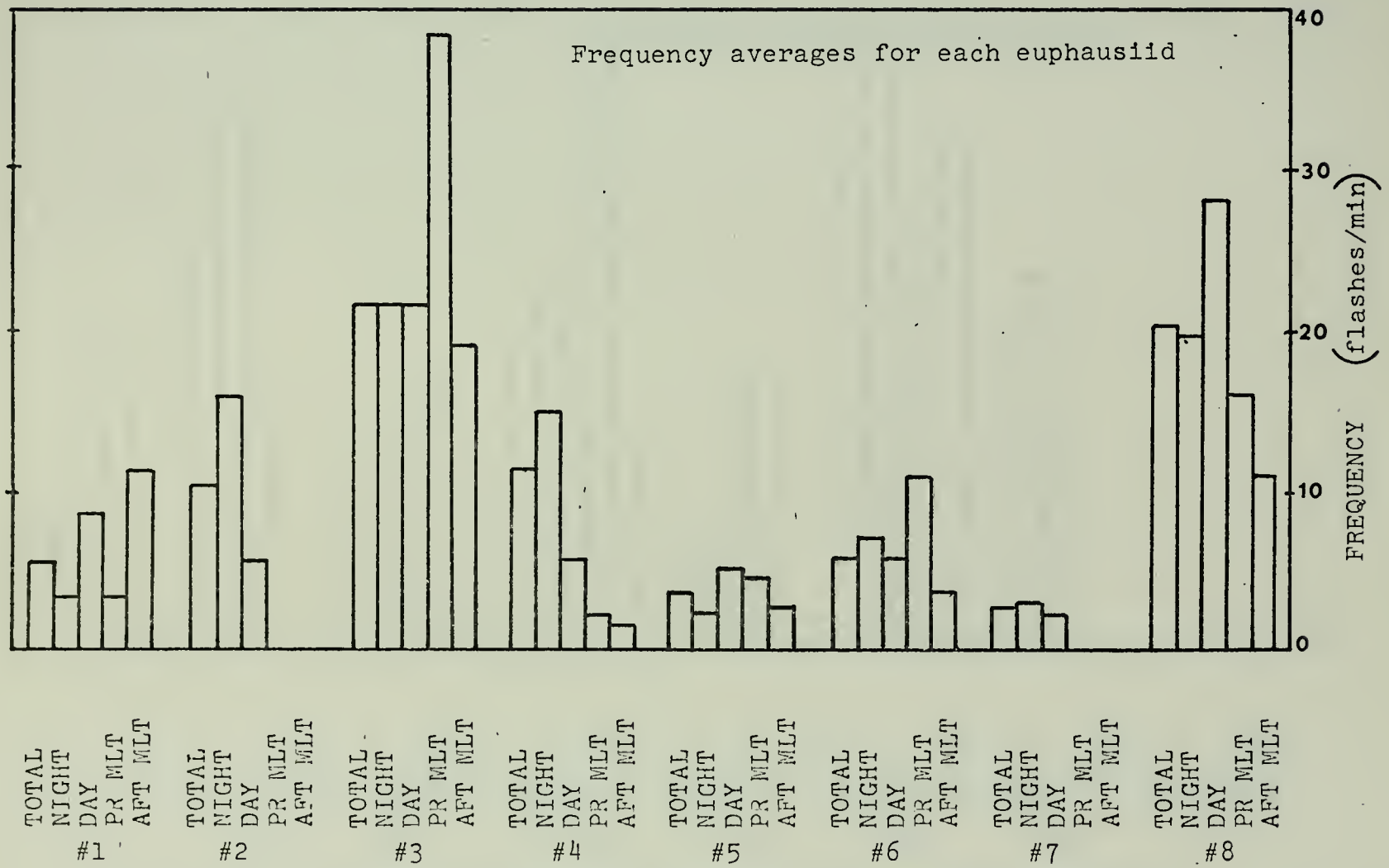


Figure 45. Individual euphausiid average for parameters indicated (PR MLT implies "prior to moulting". AFT MLT implies "after moulting").

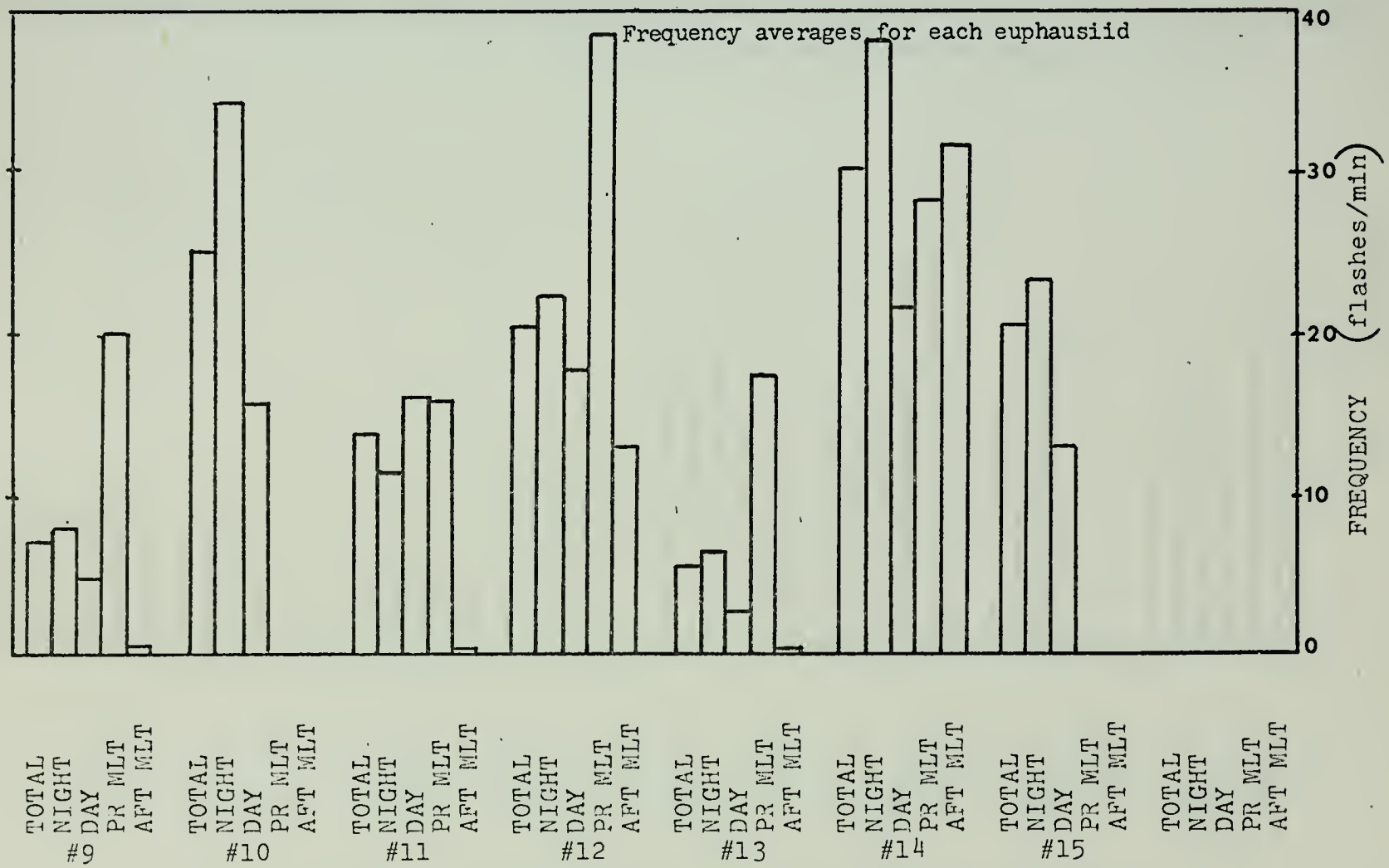


Figure 46. Individual euphausiid average for parameters indicated (PR MLT implies "prior to moulting". AFT MLT implies "after moulting").

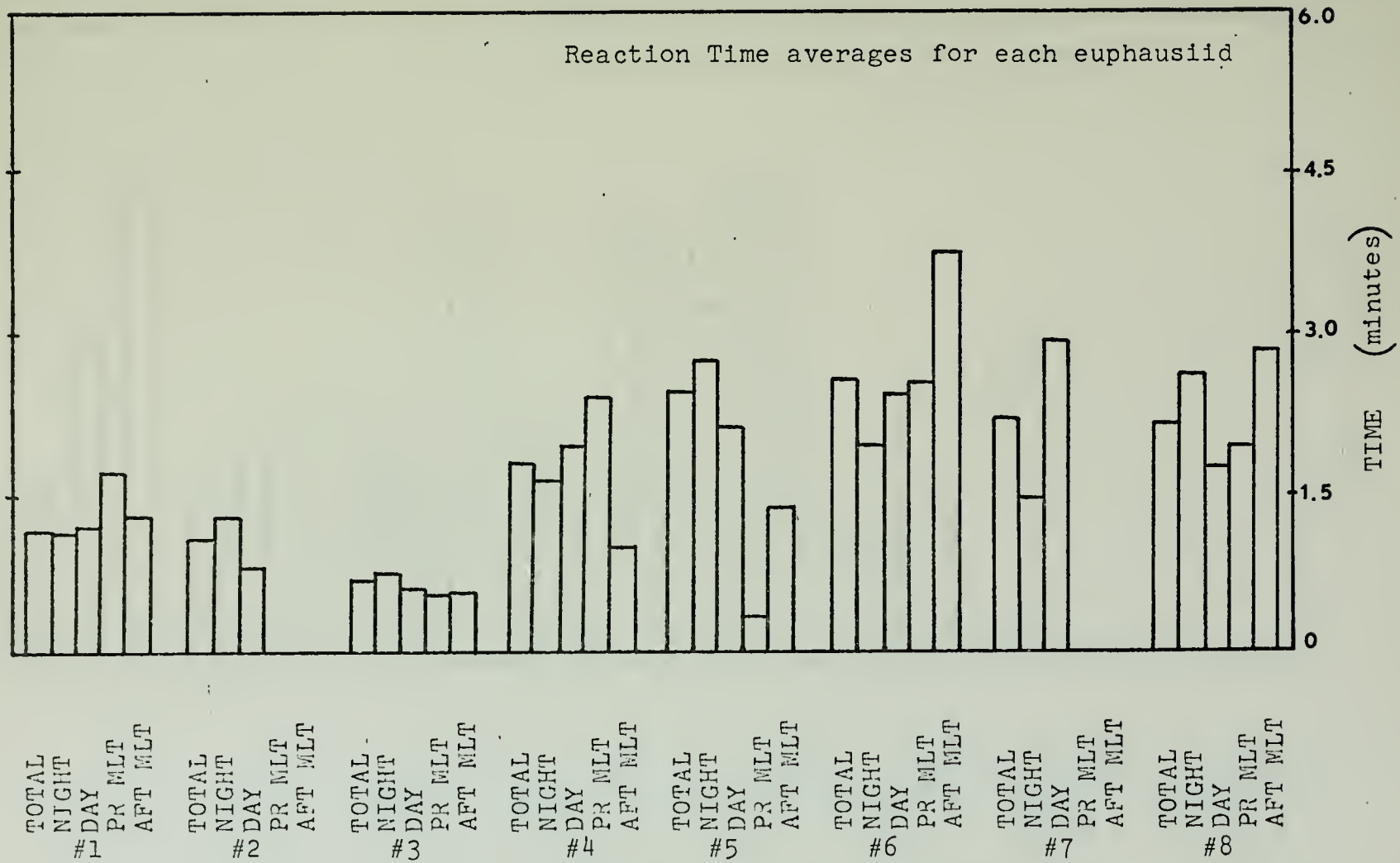


Figure 47. Individual euphausiid average for parameters indicated (PR MLT implies "prior to moulting". AFT MLT implies "after moulting").

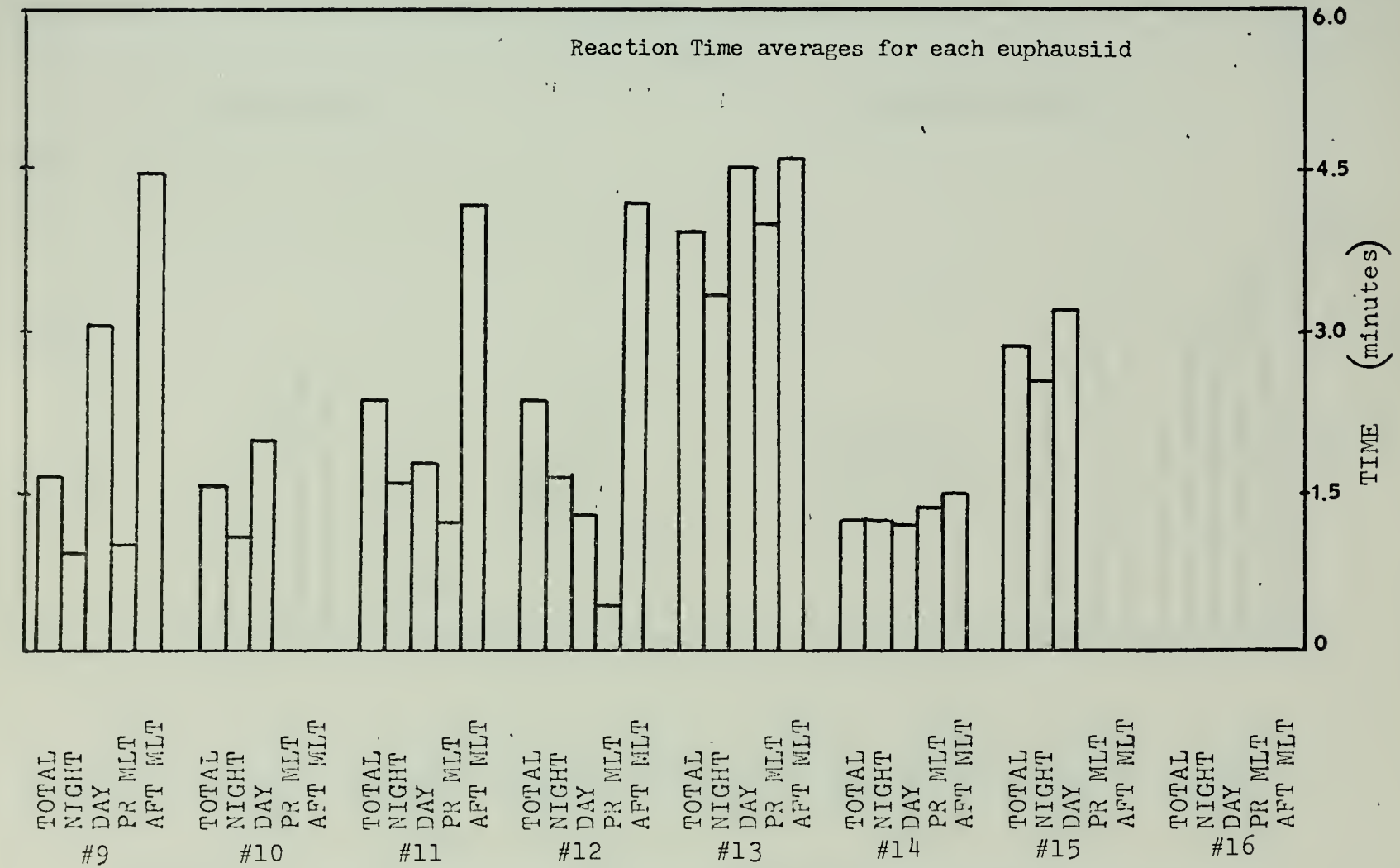


Figure 48. Individual euphausiid average for parameters indicated (PR MLT implies "prior to moulting". AFT MLT implies "after moulting").

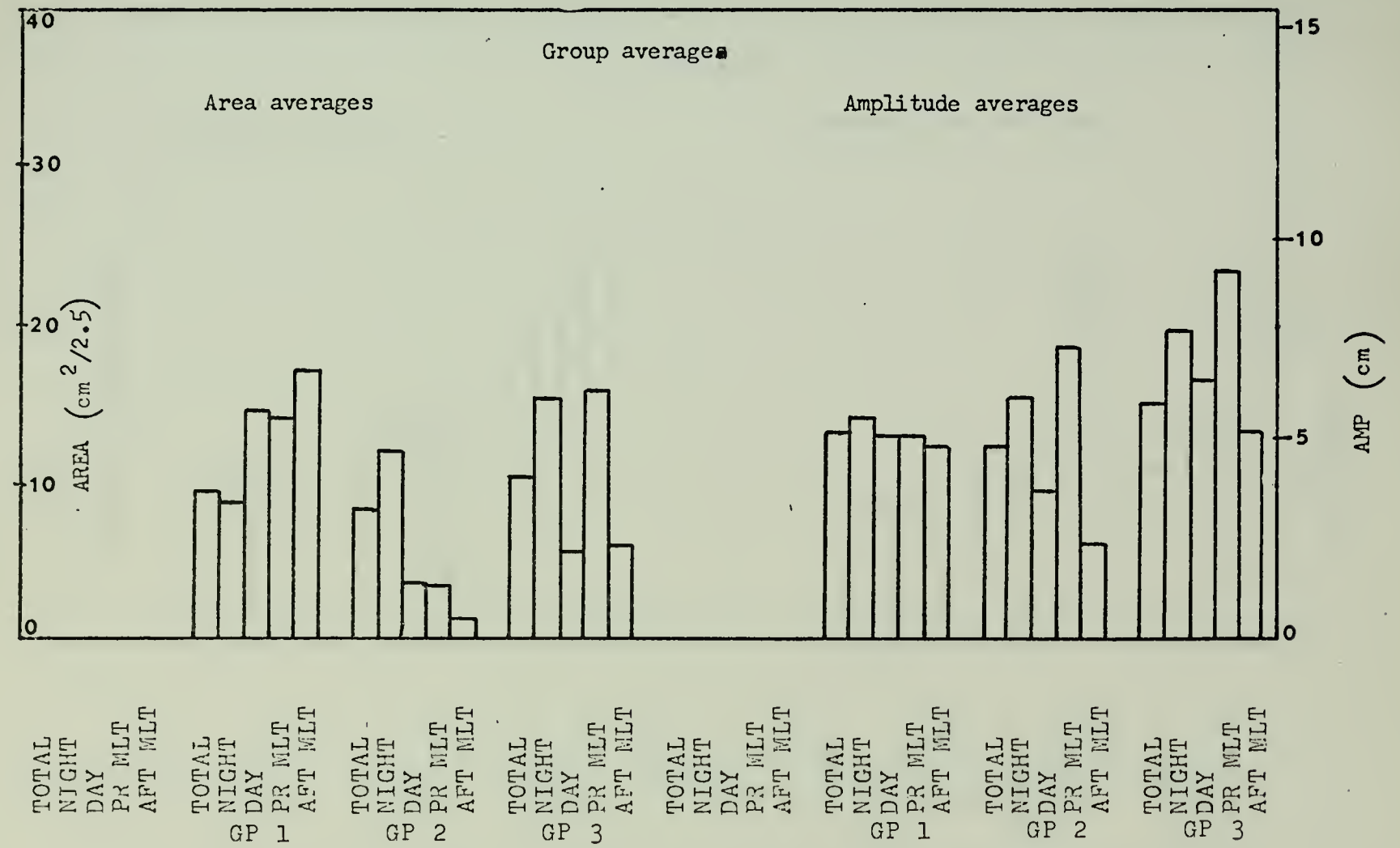


Figure 49. Group averages by temperature group for parameters indicated (PR MLT implies "prior to moulting". AFT MLT implies "after moulting").

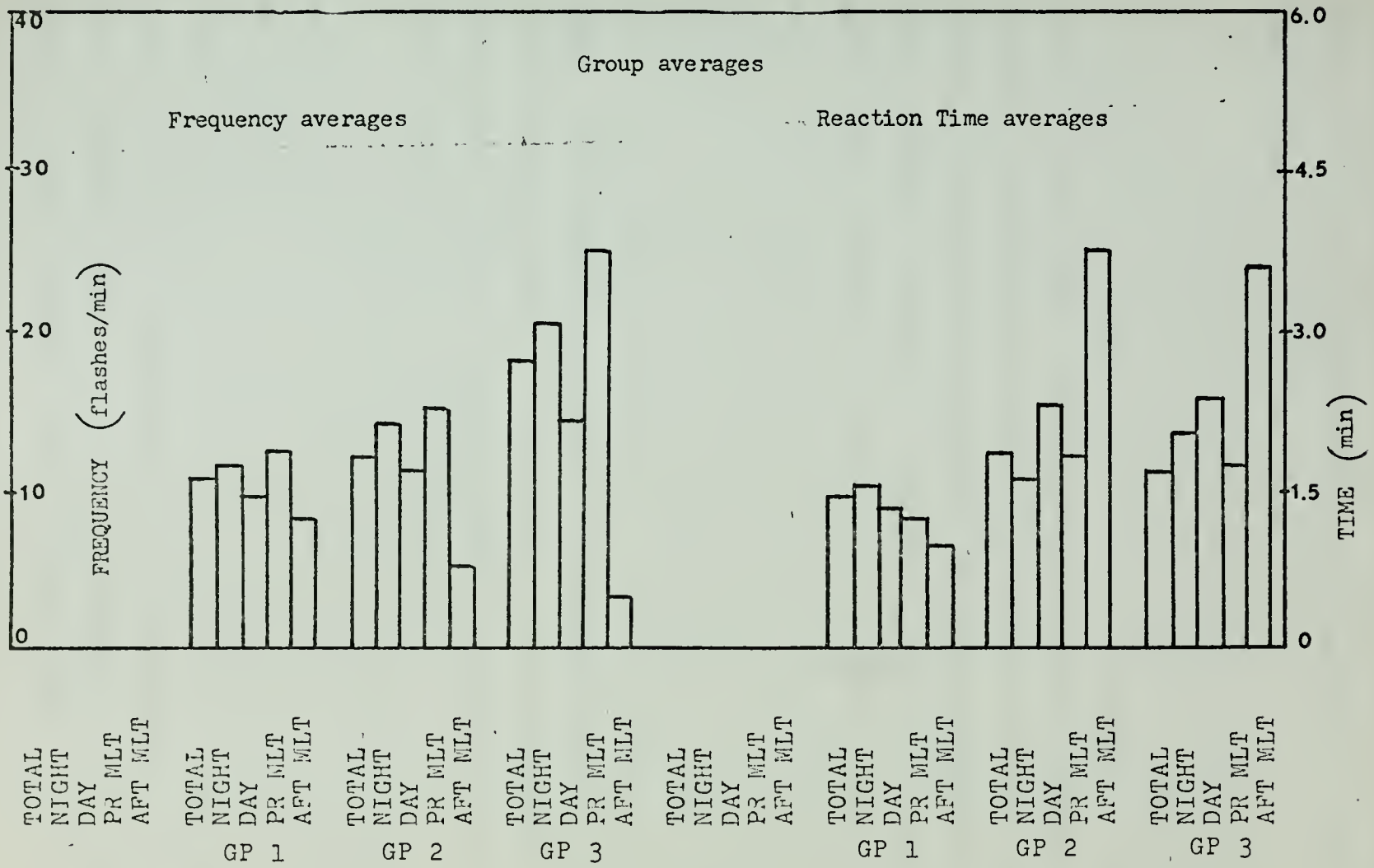


Figure 50. Group averages by temperature group for parameters indicated (PR MLT implies "prior to moulting". AFT MLT implies "after moulting").

average maximum pulse amplitude at midnight than at noon; and ten of fifteen greater average flash rates at midnight than at noon. The reaction times averaged lower at night than at noon for six of the fifteen.

To test the effects of moulting upon bioluminescent activity comparisons were made between the tests prior to moulting and the tests made just after moulting. These two test periods were then compared with total average activity. The average activity indicated by the test just prior to moulting was much greater than that indicated by the test just after moulting. A comparison of "prior to moulting" versus "after moulting" activity shows that eight of eleven euphausiids displayed more light output, eight of eleven displayed greater average maximum amplitude and one the same amplitude and ten of eleven displayed greater average flash rates before than after moulting. The reaction times were lower on the average just prior to moulting than just after for nine of the eleven. (Only eleven of the euphausiid records were used in moulting averages, as there was not enough data for four of the euphausiids.)

Comparisons of tests made just prior to moulting with the total average activity shows that eleven euphausiids displayed more light output, eight of the eleven displayed greater average maximum amplitude and greater average flash rates just prior to moulting than at other times. For the after moulting activity versus total average activity: only four of eleven displayed greater average light output and

greater maximum amplitude, and two of eleven euphausiids displayed greater average flash rates. This seems to indicate greater average bioluminescent activity (under artificial stimulation) by the euphausiids just prior to moulting and less than average activity after moulting.

The group averages show the effect that temperature (assuming that all other variables were actually the same for all groups) has on the measured parameters. The activity at midnight is greater than at noon for all three temperature groups for the parameters of average light output, average maximum amplitude, and average flash rate with one exception. Group one, which was maintained at 12°C, had a greater average light output at the noon test. The same result is true for just prior to moulting tests versus just after moulting tests for the three groups with the same exception. There was greater variation in comparing just prior to moulting tests and just after moulting tests to total average activity.

d. Sympathetic Bioluminescent Response

Mauchline (1960) indicated that there may be a sympathetic flash response, i.e. the bioluminescent response of one organism to the light flash of another. Eight euphausiids were selected, after a postive response to photoflash stimulation, to test this hypothesis and also to determine the presence, if any, of a male-female response such as found in the firefly, Photinus pyralis (McElroy and Seliger, 1962).

The euphausiids were placed in individual 100-ml beakers and seven were placed in a circle in a water bath. The eighth euphausiid was photoflash stimulated and placed in the center of the circle. The circle was about twelve inches in diameter and the center euphausiid about six inches from each of the others. The bioluminescence was clearly visible to all other euphausiids due to the swimming patterns of the stimulated euphausiid.

The euphausiids were kept in marked containers and each in turn was stimulated and placed in the center. They were allowed to rest for one hour between tests. All euphausiids responded positively to photoflash stimulation, i.e. they bioluminesced, but negatively to sympathetic stimulation – at least to visual observation. The observer was given ten minutes to become accustomed to the dark prior to each test. Three males and five female E. pacifica with body lengths of from 14 mm to 19 mm formed the test group.

V. DISCUSSION

A. AT-SEA RESULTS

Useable results in this study were obtained on R/V ACANIA cruises 74-14 and 74-19. Testing of equipment and equipment failure combined with a limited time frame in which to gather information prevented more numerous results. The results from these two cruises are presented.

On ACANIA cruise 74-14, bioluminescent activity versus depth tests, using the sensor with the VMT, seemed to indicate that the bioluminescent flash rates of euphausiids may decrease with increases in pressure and/or decreases in temperature. Euphausiids, captured in the upper 100 meters of the water column, and exposed primarily to pressure and temperature changes exhibited significant changes in light output with depth. At depths of 100 meters and below they showed a marked decrease in bioluminescent activity (Figure 11). Kay (1966) determined in laboratory tests that the euphausiid Meganyctiphanes norvegica had increased reaction time to photoflash stimulus with decreasing temperature (two minutes at 7.5°C-12.5°C versus nine minutes at 2.5°C). The temperature change in the water column for our in situ test was from 11°C at the surface to 9.4°C at 250 meters (Appendix C). Clarke and Hubbard (1959) recorded bioluminescence to depths of 3750 meters but the effects of pressure changes on bioluminescent activity is unknown.

A second in situ test on ACANIA cruise 74-19 also yielded decreased bioluminescent activity with increased depth although the bioluminescent activity of the environment differed somewhat. The environmental bioluminescence on ACANIA cruise 74-14 decreased much more rapidly with depth. This is depicted in Figure 51. The flash rates for both the test organisms and the surrounding environment are plotted against depth for ACANIA cruise 74-14 and cruise 74-19. ACANIA cruise 74-14 tests were made two hours later in the evening than for cruise 74-19. The later tests (cruise 74-14) show a greater concentration of bioluminescent organisms nearer the surface, probably because a greater percentage of DSL organisms had completed migration in this case. The euphausiid activity depicted is similar in profile to the environmental activity but is dependent upon flash rates of a fixed number of organisms.

The decrease in flash rate by the euphausiids could be a defense mechanism (Tett and Kelly, 1973) as the organism is lowered in the water column out of the upper 100 meters where caught.

Euphausiids were exposed to outside environmental light as a test on ACANIA cruise 74-19 to determine possible effects of environmental light on euphausiid light output. The results of this test indicated a slight gain in flash rate when exposed to other bioluminescence. Positive response could indicate a sympathetic type reaction, species recognition, or a male-female response (Tett and Kelly, 1973). (Laboratory tests in this study yielded no intraspecies stimulus — see

B. this section.) This activity is also plotted on Figure 51 and depicts the higher activity displayed under these test conditions.

Some investigators (Clarke, 1960; Tett, 1973) suggest that once sufficient background studies have been made a record of the intensity a flash as a function of time or flash "signature" may prove a useful organism identifier. Figure 52a is a bioluminescent background (outside VMT) record of the environment at 100 meters depth recorded at a speed of eight inches per minute. Figure 52b is the bioluminescent record of 10 euphausiids at 50 meters at the same recording speed. Figures 45c and 45d display large E. pacifica (in VMT) flashes at 100 meters, probably individual flashes, at two inches per minute recording speed. Laboratory recordings of individual E. pacifica (swimming in a 60 ml beaker) are depicted in Figures 52e and 52f. The laboratory euphausiids had been flash stimulated and sustain activity over a much longer time period.

These records indicate that although an individual order or species of animal may have an individual signature, its extraction from a record of the environmental bioluminescence may prove difficult. This is primarily due to the presence of background light. If this is to be a useable tool the animals will have to be tested individually in situ to obtain a true signature.

There is not enough evidence to suggest that euphausiids have a diurnal rhythm of luminescence or that they tend to

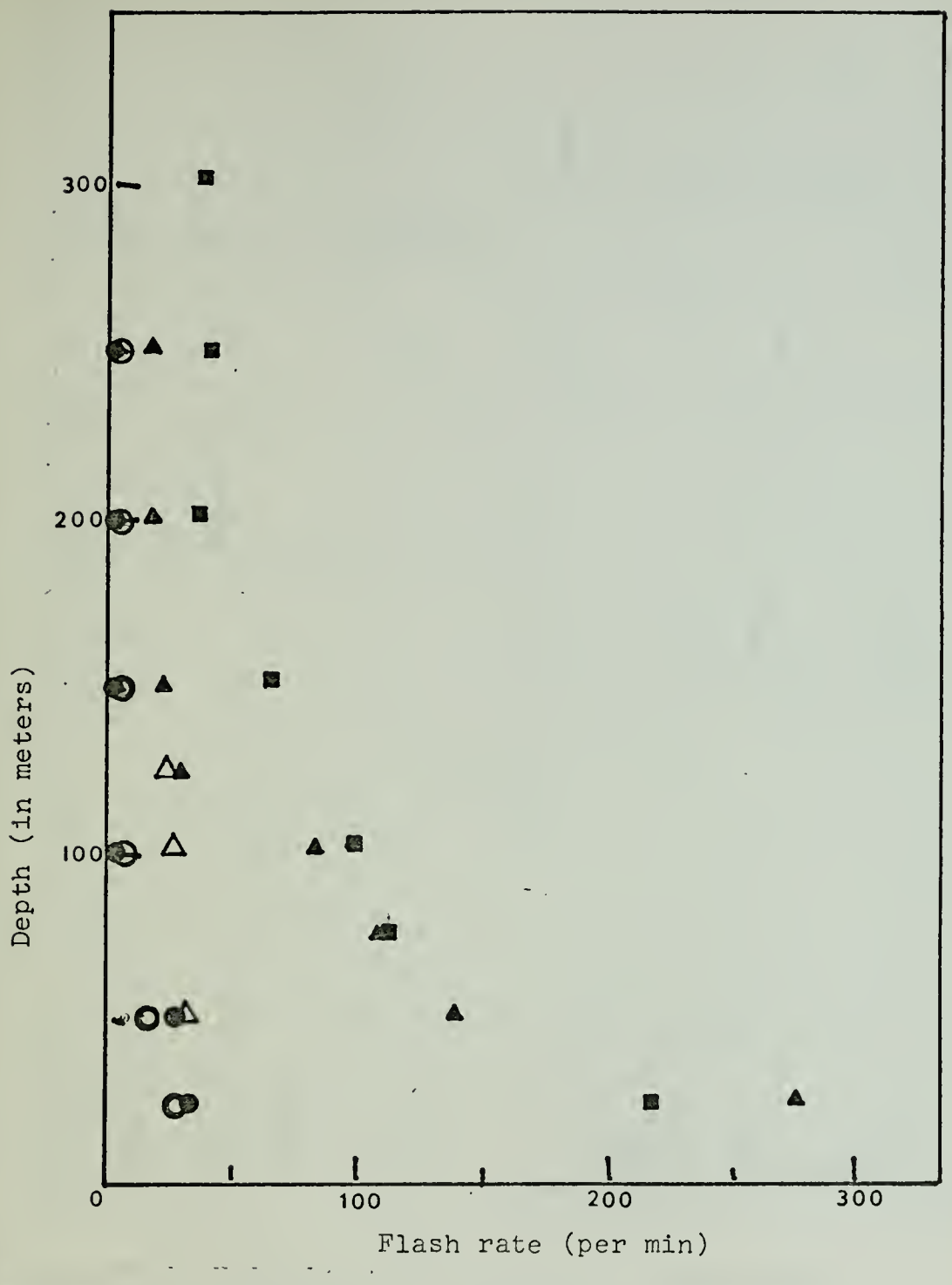


Figure 51. Chart of bioluminescent activity versus depth of environment and test groups of euphausiids for ACANIA cruises 74-14, 74-19.

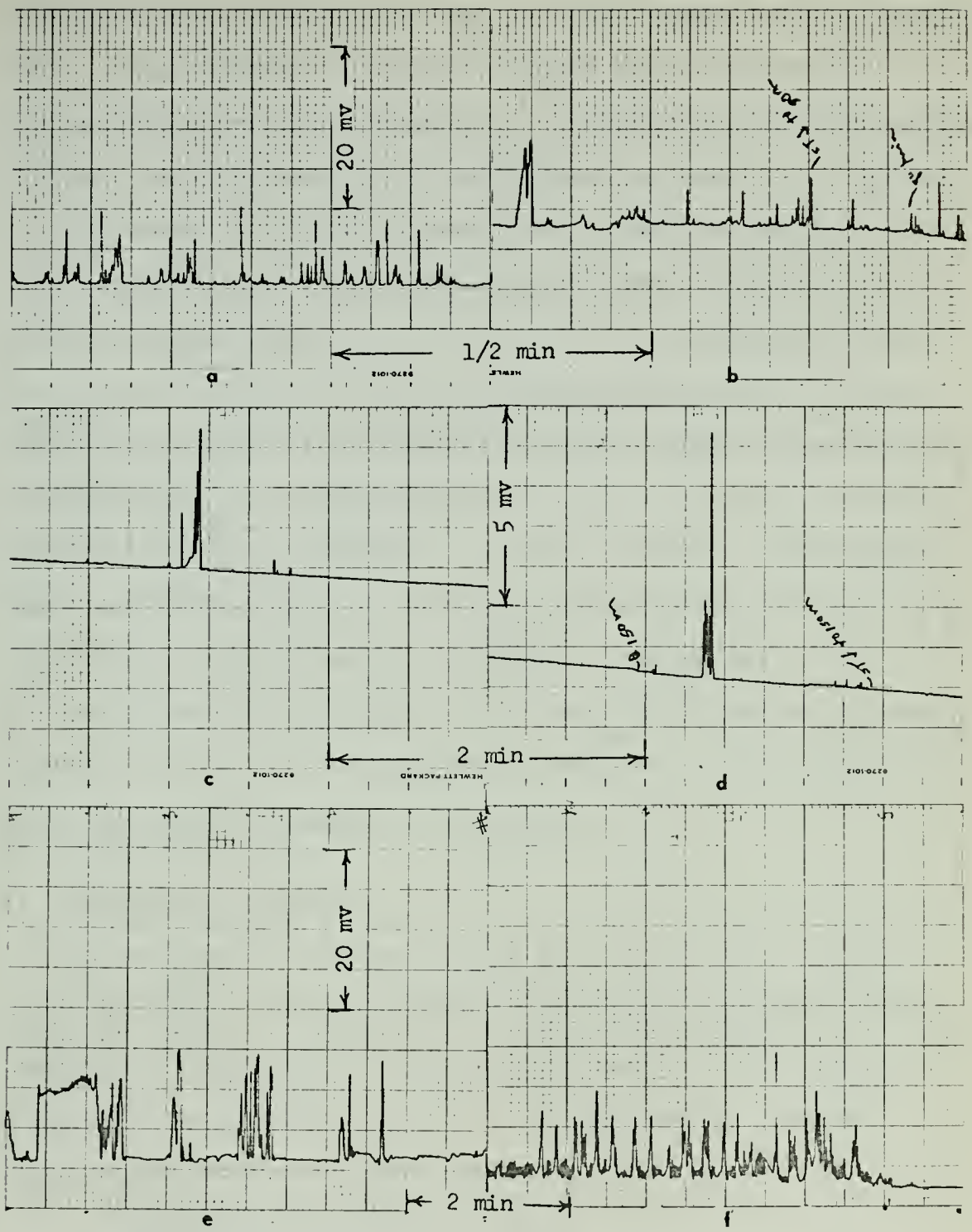


Figure 52. Bioluminescent records of environment and individual E. pacifica.
a. Environment at 100 m depth
b. 10 euphausiids at 50 m in VMT
c,d. Large euphausiid flashes at 100 m in VMT
e,f. Laboratory recordings of light output of individual E. pacifica.

bioluminesence more at dusk and at dawn (Mauchline and Fisher, 1969). The answers to these questions are important in trying to determine the function of euphausiid bioluminescence and what natural levels of luminescence to expect in migrating or stationary layers of euphausiids. (This reasoning could also be applied to other bioluminescent DSL organisms.) If bioluminescent activity is greatest during migration it may be used for maintaining spacing between individuals during migration, species identification during migration and keeping continuity in the migrating layer, or for separation between layers of different animals to prevent mixing of the layers (Tett and Kelly, 1973). The photomultiplier sensor used with the VMT may provide answers to some of the above.

There are no field tests of in situ activity of bioluminescence with which to compare our results and the number of tests run require guarded conclusions.

B. LABORATORY RESULTS

The results of the laboratory tests from this study are presented here. The reader is again reminded (Chapter II) that there are many unknowns in this type of testing and in trying to relate the results to the behavior of the test organism in its natural environment.

1. Results of Spontaneous Tests

Mauchline (1960) in tests for spontaneous bioluminescence on the euphausiid, Meganyctiphanes norvegica, and Tett (1972) in similar tests on M. norvegica and

Thysanoessa raschii determined that this type of activity is rare and unpredictable in the laboratory. However most of these animals die within a few days after capture, demonstrating inability to live in the artificial laboratory environment. E. pacifica has demonstrated a much longer life expectancy in the lab – on the order of months (Lasker and Theilacker, 1965; Komaki, 1966). Five tests were run in the present study to try to relate spontaneous activity to time of day. If a pattern were determinable it might yield information on the functions of bioluminescence.

Examination of the records of the activity of the five groups did not reveal any identifiable pattern (Figure 15). The euphausiids were active for periods which varied from 100% of the time in a ten-hour test group (Group 3) to 20% of the time in a 34 hour test group (Group 5). A feature of all of the spontaneous activity was the low amplitude (0.1 mv, see Figure 18) response indicating weak flashes or possibly a soft glow type bioluminescence exhibited on several occasions by euphausiids while under visual observation. The initial stimulus, caused by handling the euphausiids when moving them to the test equipment, lasted 15 to 20 minutes and was of high (up to 2.5 mv) amplitude.

2. Results of Bioluminescent Activity Versus Ambient Light

The ambient light levels for E. pacifica in its own environment is about 10^{-4} $\mu W/cm^2$ (Kampa and Boden, 1954). Tests to determine the effects of different light levels on

the bioluminescent activity of laboratory euphausiids were run on a large (25) group of E. pacifica. A relatively bright light did seem to cause a faster loss of bioluminescent activity. The other light intensity categories; "dark", "dim", and alternating "dark and dim" were inconclusive, A concurrent moulting test was also inconclusive. An important result of this study was the development of new laboratory test equipment and methods for further study.

3. Results of Bioluminescent Activity Versus Temperature, Moulting and Time of Day.

If the euphausiids use bioluminescence for some specific function, i.e. feeding in the near surface water, they might exhibit more activity in the laboratory under stimulation, during that time period they would normally have been feeding. The euphausiids were tested for a relative bioluminescent activity at noon and at midnight. It was suspected that at these two periods corresponding to feeding at the surface or resting at depth the greatest difference in activity would occur. Another possible period of great activity was that of migration. This had the disadvantage that if tested against a non migration time period, equal time periods between tests would be difficult to set up and therefore might bias the results.

The results of the tests indicate that two-thirds of the euphausiids showed a greater response to stimulation at the midnight test than at the noon test. When the individual results were averaged within their respective temperature

groups, the preference for night over day was more pronounced. Therefore, excluding effects of pressure and other parameters and time periods not tested, euphausiids appear to demonstrate greater use of the bioluminescent function during the night, near-surface period of their diurnal cycle.

The results of bioluminescent activity during moulting periods indicated that the average activity in the tests prior to moulting was greater than the average activity in the tests after moulting by a large percentage. This would be the expected result, as other organisms normally have a quiescent period following moulting. This same relation was evident in comparing the time of moulting with average activity and in the group comparison tests.

C. GENERAL DISCUSSION AND FUTURE USE OF VMT

The photomultiplier light detector is a valuable tool in the study of underwater light but has been limited in some aspects of its use (Boden and Kampa, 1964; Mauchline and Fisher, 1969; Tett and Kelly, 1973). Work in marine bioluminescence studies has lost impetus in recent years (Tett and Kelly, 1973), and it is felt that this may be due, in part, to the slow progress in underwater light detector methodology. The VMT utilized with the underwater light sensor introduces a new approach to in situ studies. If laboratory tests are run in conjunction with in situ studies, further insight into the functions of marine bioluminescence will undoubtedly be gained.

Three of the major problems encountered in studies of marine bioluminescence using the photomultiplier light detector are solved in part by use of the VMT with the sensor. A specific organism can be studied in situ; but it is physically restrained to a certain volume of that environment. The distance and angle from the organism to the light detector are maintained within a given restricted range. The artificial stimulus due to the vertical oscillation of the sensor in the water column is decreased to some degree. The physical stirring and overpressure caused by the sensor's movement through the water are absent, but other factors, such as cable vibration carried to the VMT, may still cause some stimulation.

Euphausiids could be easily maintained and monitored through a 24 hour period in the VMT. A suitable high frequency fathometer could be used to moniter DSL depths and keep the VMT within the DSL. The bioluminescent activity could then be monitored through a 24 hour cycle. The apparatus could also be used to test for bioluminescent changes in periods of euphausiid swarming, for near-surface activity (feeding), for at-depth activity, and for changes with time, light and other parameters. The VMT can be used to hold organisms for testing within their own environment with little change to that environment, or it may be used to partially control certain of the physical and chemical elements of the environment. Light may be fully or partially masked out. The small access holes can be enlarged and

increased in number (or a flow-through system included) to allow oxygen, pH, salinity and nutrient changes to occur more rapidly within the tube. The tube (or a shorter version) could be filled with water from selected depths to vary the parameters to other levels of the water column.

VI. CONCLUSIONS

1. An underwater test chamber to retain organisms for testing within their own environment was designed and constructed. This chamber, called a vertical migration tube (VMT), allows partial control of the physical and ecological factors of the organism's environment during testing.

2. The use of the vertical migration tube with a photomultiplier sensor may prove to be a valuable tool for in situ mesopelagic studies. Initial tests of the unit demonstrated the feasibility of its use in the marine environment.

3. Three major problems associated with studies of marine bioluminescence using an underwater photomultiplier light detector are resolved in part by the use of the VMT with this sensor. With it a specific (known) organism can be studied in situ; the distance and angle from the organism to the sensor can be controlled; and the artificial stimulus due to the vertical oscillation of the sensor in the water column is minimized.

4. Euphausiids taken in the upper 100 meters of the water column at night decrease their bioluminescent flash rates when lowered in the water column and exposed primarily to pressure and temperature changes. This could imply that a defensive mechanism or a feeding mechanism is at work.

5. There may be an increase in euphausiid bioluminescent flash rates when stimulated by other bioluminescent organisms. This could be an intraspecies response and be either a sympathetic type reaction, species recognition, or a male-female type response. An interspecies response may be responsible also and used to provide species exclusion.

6. The laboratory test for 12- to 24-hour activity rhythms were inconclusive. Low amplitude bioluminescence was a feature of all the spontaneous activity, and this type of bioluminescence may be the "normal" light output of the euphausiid. Bright light or mechanical stirring (artificial laboratory stimulus) elicited much higher amplitude activity.

7. Laboratory test equipment was designed and built to allow a quantitative measurement to be made of the light output of a small marine organism. This equipment when used with a photomultiplier light detector and recorder allows measurements of total light output, amplitude and flash frequency to be made.

8. The use of a flash "signature" (flash amplitude as a function of time) to identify a species or an order of marine organisms, although possible, may prove difficult due to the presence of other background light.

9. A "bright" light laboratory environment seemed to cause a faster loss of bioluminescent activity in a group of test euphausiids. The ambient light level of their normal environment is on the order of 10^{-4} $\mu W/cm^2$. It was

concluded that a "dim" light environment (10^{-3} $\mu W/cm^2$) was best to maintain the euphausiids in the laboratory.

10. Laboratory tests indicated a greater bioluminescent response to a standard flash stimulus during midnight tests as opposed to noon tests. This may indicate that the euphausiid's "normal" use of bioluminescence occurs at that time. This might be associated with feeding or another surface related function.

11. Laboaratory tests of bioluminescent activity indicated greater than average response to a photoflash stimulus just prior to moulting and less than average response just after moulting. This would appear to be a normal physiological condition.

TABLE I

Record of the measured parameters of the daily bioluminescence of each E. pacifica

DATE	TOTAL LIGHT OUTPUT AREA	MAX FLASH AMP	FLASH FREQ	REACTION TIME	MOULT
		Euphausia pacifica #1:			
13 Feb n	8.0	7.8	19	0.6	M
13 Feb m	1.0	3.0	3	1.9	
16 Feb n	9.4	7.0	10	1.1	
16 Feb m	3.8	3.0	7	0.3	
17 Feb n	0.8	0.5	1	1.4	
17 Feb m	0.6	0.3	1	0.3	
18 Feb n	0.4	1.9	3	1.7	
18 Feb m	5.6	4.5	3	2.0	M
		Euphausia pacifica #2:			
13 Feb n	1.3	4.8	6	0.8	
13 Feb m	5.9	6.7	16	1.3	
		Euphausia pacifica #3:			
13 Feb n	45.2	13.4	32	0.3	
13 Feb m	40.9	13.4	35	0.6	
16 Feb n	33.8	13.4	32	0.5	
16 Feb m	32.2	13.4	39	0.5	
17 Feb n	62.2	13.4	19	0.5	M
17 Feb m	13.3	12.7	13	0.8	
18 Feb n	16.1	5.5	5	1.0	
18 Feb m	1.8	0.5	1	1.1	

TABLE I (continued)

DATE	TOTAL LIGHT OUTPUT AREA	MAX FLASH AMP	FLASH FREQ	REACTION TIME	MOULT
		Euphausia pacifica #4:			
13 Feb n	1.2	2.3	2	0.9	
13 Feb m	1.8	2.3	4	1.2	
16 Feb n	1.7	0.5	2	5.3	
16 Feb m	32.5	13.4	50	1.6	
17 Feb n	14.2	13.4	17	0.6	
17 Feb m	0.7	2.5	2	2.4	
18 Feb n	0.4	0.1	1	1.0	M
18 Feb m	8.1	6.3	4	1.2	
		Euphausia pacifica #5:			
13 Feb n	0.6	0.6	1	1.1	M
13 Feb m	1.5	1.5	1	2.2	
16 Feb n	4.5	3.5	13	2.1	
16 Feb m	3.3	4.9	5	0.3	
17 Feb n	4.2	4.6	4	1.6	
17 Feb m	0.2	1.1	2	3.6	
18 Feb n	0.7	1.1	2	3.6	
18 Feb m	0.5	0.8	2	5.0	

TABLE I (continued)

DATE	TOTAL LIGHT OUTPUT AREA	MAX FLASH AMP	FLASH FREQ	REACTION TIME	MOULT
		Euphausia pacifica #6:			
13 Feb n	1.1	1.1	2	1.8	
13 Feb m	4.3	3.7	7	2.1	
16 Feb n	3.3	1.3	9	3.7	M
16 Feb m	30.9	13.4	18	0.3	
17 Feb n	10.9	7.5	19	1.3	
17 Feb m	2.4	3.8	6	1.8	
18 Feb n	1.3	2.0	4	3.0	
18 Feb m	15.7	13.4	18	1.1	
22 Feb m	0.1	0.1	0	0.7	M
23 Feb n	0.1	0.1	0	0.9	
24 Feb n	1.2	1.1	7	3.2	
25 Feb n	4.6	6.6	12	2.4	
26 Feb n	0.5	0.7	1	2.9	
27 Feb n	6.8	6.1	23	1.6	
28 Feb m	0.4	1.1	1	1.5	M
2 Mar n	0.2	1.3	3	2.4	
3 Mar n	2.0	1.6	3	1.9	
3 Mar m	0.0	0.0	0	6.0	
5 Mar n	1.1	1.7	3	3.8	
6 Mar n	0.0	0.0	0	6.3	M

TABLE I (continued)

DATE	TOTAL LIGHT OUTPUT AREA	MAX FLASH AMP	FLASH FREQ	REACTION TIME	MOULT
		Euphausia pacifica #7:			
13 Feb n	1.2	1.0	3	1.2	
13 Feb m	7.5	3.0	5	1.3	
16 Feb n	0.1	0.1	0	4.7	
16 Feb m	0.1	1.0	1	0.9	
17 Feb n	0.3	0.8	4	3.0	
17 Feb m	0.5	1.1	6	2.1	
18 Feb n	0.0	0.0	0	6.0	
18 Feb m	0.0	0.0	0	6.0	M

TABLE I (continued)

DATE	TOTAL LIGHT OUTPUT AREA	MAX FLASH AMP	FLASH FREQ	REACTION TIME	MOULT
		Euphausia pacifica #8:			
13 Feb n	17.2	13.4	41	0.7	M
13 Feb m	23.1	13.4	50	0.6	
16 Feb n	32.3	13.4	41	1.3	
16 Feb m	7.5	5.0	34	0.8	
17 Feb n	4.1	5.5	53	1.3	
17 Feb m	7.2	9.1	11	1.2	
18 Feb n	10.2	13.4	17	0.9	
18 Feb m	9.7	12.6	24	1.0	M
22 Feb m	0.1	0.2	0	6.0	
23 Feb n	0.5	1.1	7	4.1	
24 Feb n	5.1	10.1	29	2.1	
25 Feb n	2.5	6.3	9	1.6	M
26 Feb n	10.2	12.2	18	1.3	
27 Feb n	1.8	5.1	14	2.0	
28 Feb m	0.0	0.0	0	6.0	
2 Mar n	0.7	2.1	5	2.3	
3 Mar n	0.6	1.3	2	2.7	
3 Mar m	0.0	0.0	0	6.0	M

TABLE I (continued)

DATE	TOTAL LIGHT OUTPUT AREA	MAX FLASH AMP	FLASH FREQ	REACTION TIME	MOULT
		Euphausia pacifica #9:			
13 Feb n	2.7	1.0	1	3.5	
13 Feb m	50.7	13.4	8	0.3	
16 Feb n	15.3	11.6	6	0.6	
16 Feb m	43.7	13.4	10	0.3	
17 Feb n	0.7	3.5	4	2.0	
17 Feb m	10.7	12.5	10	0.6	
18 Feb n	2.5	9.2	10	1.0	
18 Feb m	0.4	1.4	2	1.8	
22 Feb m	1.1	4.2	4	0.3	M (19th)
23 Feb n	0.5	1.0	6	1.8	
24 Feb n	12.4	13.4	23	0.4	
25 Feb n	3.0	0.7	1	3.9	M
26 Feb n	3.0	3.4	6	3.0	
27 Feb n	3.4	3.6	14	1.8	
28 Feb m	0.6	3.5	4	1.4	
2 Mar n	0.2	0.6	1	3.4	
3 Mar n	1.3	3.2	2	1.6	
3 Mar m	3.5	0.4	18	1.6	
5 Mar n	0.0	0.0	0	6.3	M

TABLE I (continued)

DATE	TOTAL LIGHT OUTPUT AREA	MAX FLASH AMP	FLASH FREQ	REACTION TIME	MOULT
		Euphausia pacifica #10:			
13 Feb n	3.6	3.8	23	1.0	M
13 Feb m	7.9	3.8	26	1.0	
16 Feb n	11.8	9.1	22	2.1	
16 Feb m	58.9	13.4	58	0.3	
17 Feb n	1.7	1.8	3	2.8	
17 Feb m	26.2	12.5	19	1.8	
		Euphausia pacifica #11:			
16 Feb m	15.7	13.4	32	1.3	
17 Feb n	11.7	12.4	21	0.6	
17 Feb m	3.3	5.1	14	1.2	
18 Feb n	5.2	5.6	19	0.7	
18 Feb m	2.3	6.7	16	1.2	
22 Feb m	1.6	4.5	3	1.9	M
23 Feb n	0.6	1.2	0	4.2	
24 Feb n	2.2	5.1	37	2.0	
25 Feb n	1.9	13.4	11	0.9	
26 Feb n	0.5	0.2	1	1.3	
27 Feb n	7.3	10.2	19	1.1	
28 Feb m	0.1	0.5	1	2.4	
2 Mar n	0.0	0.0	0	6.0	
3 Mar n	1.3	2.6	3	2.0	
3 Mar m	0.6	1.3	2	1.5	
5 Mar n	0.3	1.1	2	4.0	
6 Mar n	0.4	0.8	1	3.2	

TABLE I (continued)

DATE	TOTAL LIGHT OUTPUT AREA	MAX FLASH AMP	FLASH FREQ	REACTION TIME	MOULT
		Euphausia pacifica #12:			
16 Feb m	32.4	13.4	45	0.5	
17 Feb n	8.9	11.5	40	1.3	
17 Feb m	21.3	12.3	86	1.8	
18 Feb n	21.7	13.4	20	1.2	
18 Feb m	40.9	13.4	.39	0.4	
22 Feb m	7.7	11.0	13	4.2	M
23 Feb n	2.9	4.7	9	0.9	
24 Feb n	2.3	3.5	6	3.4	
25 Feb n	0.6	2.3	2	2.4	
26 Feb n	3.7	6.6	13	1.5	
27 Feb n	4.6	2.0	11	0.5	
28 Feb m	9.3	11.5	16	0.3	
2 Mar n	0.7	1.8	3	0.8	
3 Mar n	13.8	13.4	22	0.4	
3 Mar m	4.3	7.2	4	2.1	
5 Mar n	3.8	4.8	6	1.5	
6 Mar n	1.1	1.4	1	3.5	M

TABLE I (continued)

DATE	TOTAL LIGHT OUTPUT AREA	MAX FLASH AMP	FLASH FREQ	REACTION TIME	MOULT
		Euphausia pacifica #13:			
16 Feb m	24.6	12.6	17	1.5	
17 Feb n	6.0	2.4	3	1.3	
17 Feb m	1.3	1.5	0	3.8	
18 Feb n	2.9	2.1	0	4.7	
18 Feb m	10.8	7.6	18	4.0	
22 Feb m	0.7	0.0	0	4.6	M
23 Feb n	0.0	0.5	0	6.0	
24 Feb n	0.0	0.1	8	6.0	

TABLE I (continued)

DATE	TOTAL LIGHT OUTPUT AREA	MAX FLASH AMP	FLASH FREQ	REACTION TIME	MOULT
		Euphausia pacifica #14:			
16 Feb m	33.9	13.4	43	0.4	
17 Feb n	20.0	10.1	50	0.5	
17 Feb m	13.7	5.7	60	1.4	
18 Feb n	3.1	4.4	14	1.4	
18 Feb m	18.5	8.6	45	1.3	
22 Feb m	27.3	13.4	57	1.5	M
23 Feb n	3.6	13.4	30	1.2	
24 Feb n	5.6	13.4	16	1.2	
25 Feb n	22.7	13.4	31	1.1	
26 Feb n	13.5	13.4	26	0.8	
27 Feb n	6.6	13.4	12	1.3	
28 Feb m	9.0	6.1	7	1.4	M
2 Mar n	2.9	3.7	23	1.8	
3 Mar n	8.6	13.4	19	1.4	
3 Mar m	26.6	13.4	21	0.9	
5 Mar n	7.5	11.9	15	1.5	
6 Mar n	7.4	13.4	22	1.6	
		Euphausia pacifica #15:			
16 Feb m	49.9	13.4	43	1.2	
17 Feb n	21.6	12.2	25	0.4	
17 Feb m	2.2	2.2	4	3.8	
18 Feb n	0.8	1.1	1	6.0	
18 Feb m		no test			M

TABLE II

Averages of Each of the Measured Parameters of the Daily Biluminescent Activity of E. Pacifica

E. pacifica	AVG	NIGHT	DAY	BEFORE MOULT	AFTER MOULT
#1					
Total Light Output (Area)	3.7	2.75	4.65	0.4	6.8
Max. Amp.	3.5	2.7	4.3	1.9	3.75
Flash Freq.	5.8	3.5	8.15	3.3	11.05
Reaction Time	1.16	1.12	1.2	1.7	1.3
#2					
Total Light Output (Area)	3.6	5.9	1.3		
Max. Amp.	5.75	6.7	4.8		
Flash Freq.	10.8	16.1	5.5		
Reaction Time	1.05	1.3	0.8		
#3					
Total Light Output (Area)	29.4	22.1	36.8	32.2	60.0
Max. Amp.	10.7	10.0	11.4	13.4	13.4
Flash Freq.	22.0	22.0	22.0	39.0	19.0
Reaction Time	0.66	0.75	0.57	0.5	0.5
#4					
Total Light Output (Area)	7.57	10.87	4.37	0.7	0.4
Max. Amp.	5.1	6.12	4.07	2.5	0.1
Flash Freq.	11.04	15.0	5.57	2.0	1.3
Reaction Time	1.78	1.6	1.95	2.4	1.0

TABLE II (continued)

E. pacifica	AVG	NIGHT	DAY	BEFORE MOULT	AFTER MOULT
#5					
Total Light Output (Area)	1.93	1.37	2.49	3.3	2.38
Max. Amp.	2.26	2.07	2.45	4.9	2.6
Flash Freq.	3.53	2.15	4.9	4.5	2.55
Reaction Time	2.44	2.78	2.1	0.3	1.35
#6					
Total Light Output (Area)	4.35	7.69	2.57	4.07	1.23
Max. Amp.	3.33	5.07	2.14	3.83	0.8
Flash Freq.	6.62	7.14	5.63	11.0	3.43
Reaction Time	2.53	1.93	2.14	2.5	3.73
#7					
Total Light Output (Area)	1.62	2.7	0.53		
Max. Amp.	1.7	1.67	0.63		
Flash Freq.	2.37	2.5	2.27		
Reaction Time	2.2	1.43	2.97		
#8					
Total Light Output (Area)	7.81	7.93	10.83	5.3	2.03
Max. Amp.	7.31	6.72	8.65	8.27	6.3
Flash Freq.	20.88	19.83	28.83	16.0	11.0
Reaction Time	2.11	2.6	1.72	1.9	2.87

TABLE II (continued)

E. pacifica	AVG	NIGHT	DAY	BEFORE MOULT	AFTER MOULT
#9					
Total Light Output (Area)	8.66	15.81	3.13	7.95	3.23
Max. Amp.	6.0	8.11	4.3	10.9	0.35
Flash Freq.	7.22	8.0	4.29	20.5	0.5
Reaction Time	1.62	0.9	3.05	1.0	4.5
#10					
Total Light Output (Area)	18.35	31.0	5.7		
Max. Amp.	7.4	9.93	4.9		
Flash Freq.	25.17	34.33	16.0		
Reaction Time	1.5	1.03	1.97		
#11					
Total Light Output (Area)	4.29	3.93	2.85	2.3	0.6
Max. Amp.	5.71	5.25	6.15	6.7	1.2
Flash Freq.	13.91	11.31	16.5	16.0	0.0
Reaction Time	1.67	1.58	1.67	1.2	4.2
#12					
Total Light Output (Area)	14.17	19.32	9.03	40.9	7.7
Max. Amp.	10.29	12.5	8.08	13.4	11.0
Flash Freq.	20.08	22.17	18.0	39.0	13.0
Reaction Time	1.44	1.6	1.28	0.4	4.2

TABLE II (continued)

E. pacifica	AVG	NIGHT	DAY	BEFORE MOULT	AFTER MOULT
#13					
Total Light Output (Area)	5.79	9.35	2.22	10.08	0.7
Max. Amp.	3.34	5.4	1.27	7.6	0.0
Flash Freq.	5.75	6.25	2.75	18.0	0.0
Reaction Time	3.79	3.47	4.5	4.0	4.6
#14					
Total Light Output (Area)	14.6	21.4	7.92	12.5	18.1
Max. Amp.	10.1	10.1	11.3	11.0	9.75
Flash Freq.	30.3	38.8	21.8	28.5	32.0
Reaction Time	1.18	1.2	1.17	1.3	1.45
#15					
Total Light Output (Area)	18.4	25.6	11.2		
Max. Amp.	7.22	7.8	6.65		
Flash Freq.	20.7	23.5	13.0		
Reaction Time	2.85	2.5	3.2		

TABLE III

Averages of Each Measured Parameter for Each Temperature Group of *E. Pacifica*

GROUP 1 (12°C)	AVG	NIGHT	DAY	BEFORE MOULT	AFTER MOULT
Total Light Output (Area)	9.24	8.58	9.92	9.15	17.9
Max. Amp.	5.46	5.52	5.40	5.42	4.96
Flash Freq.	10.7	11.71	9.22	12.2	8.47
Reaction Time	1.42	1.51	1.52	1.22	1.04
GROUP 2 (alt. temp. 7°C-12°C)					
Total Light Output (Area)	8.81	13.0	4.59	4.42	2.17
Max. Amp.	5.21	6.31	3.70	7.76	2.48
Flash Freq.	12.88	14.36	11.4	15.83	4.98
Reaction Time	1.91	1.58	2.42	1.8	3.7
GROUP 3 (7°C)					
Total Light Output (Area)	11.46	15.9	6.64	16.64	6.78
Max. Amp.	6.31	8.21	6.7	9.67	5.49
Flash Freq.	18.16	20.4	14.4	25.37	10.75
Reaction Time	1.64	2.07	2.38	1.72	3.61

APPENDIX A

ACANIA Cruise No.	YEAR	MONTH	DAY	Net haul (30 m) CalCOFI Station 1	XBT CalCOFI Station 1	Net haul (100 m) CalCOFI Station 3	XBT CalCOFI Station 3	*Fluorometer Study CalCOFI Station 3	Photomultiplier Sensor Test CalCOFI Station 3	Photomultiplier Sensor and VMT Test CalCOFI Station 3
73-67	73	8	1	X	X	X	X	X		
73-71			14	X	X	X	X	X		
73-78		9	6	X		X	X	X		
73-80			17	X	X	X	X		X	
73-82			25	X	X	X	X		X	
73-87		10	4	X	X	X	X		X	
73-88			9	X	X	X	X		X	X
73-90			17	X	X	X	X		X	
73-95		11	12	X		X			X	
73-96			13	X	X	X	X			
73-101		12	6	X	X	X	X		X	
74-1	74	01	9	X	X	X	X		X	X
74-6			24	X	X	X	X		X	
74-14		02	7	X		X	X		X	X
74-16			8	X	X	X	X		X	X
74-19			14	X	X	X	X		X	
74-22			22	X	X	X	X		X	X
74-30		03	11	X	X	X	X		X	

*Fluorometer studies were conducted on early cruises while the photomultiplier detector and VMT were being designed and constructed. A hose was lowered (to depths of 75 meters) and water pumped up to a surface fluorometer to measure bioluminescence.

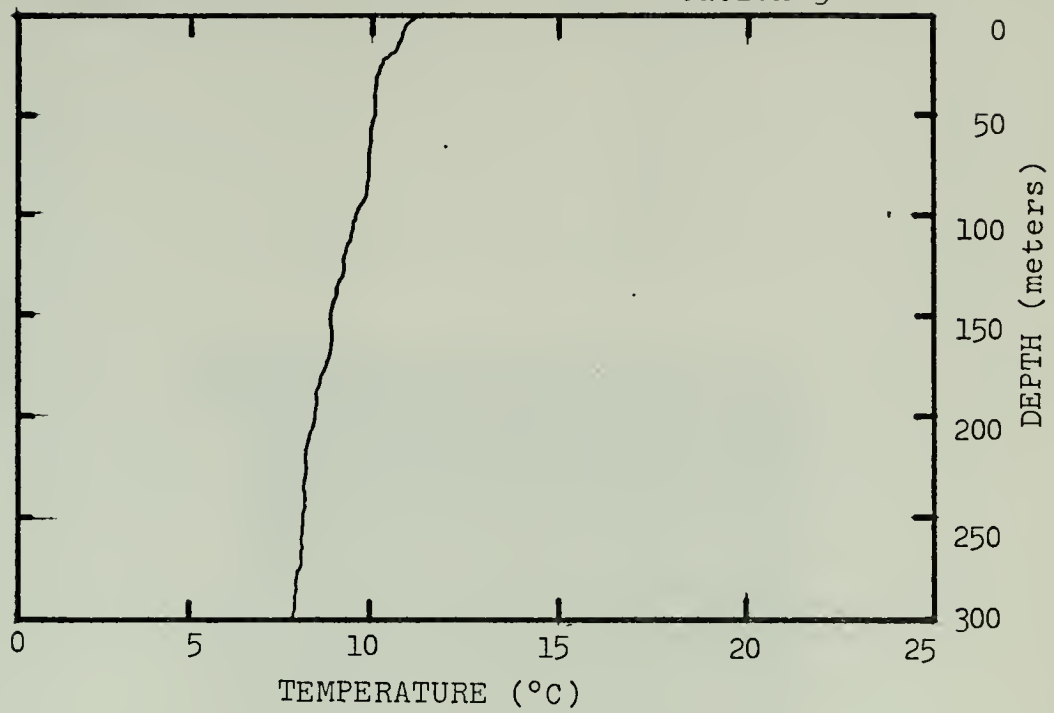
APPENDIX B

SPECIES / MONTH FOUND	August, 1973	September, 1973	October, 1973	November, 1973	December, 1973	January, 1974	February, 1974	March, 1974
Species of Euphausiid at CalCOFI Station Number Three								
Euphausia pacifica	X	X	X	X	X	X	X	X
Nyctiphanes simplex	X	X		X			X	
Thysanoessa spinifera			X					
Thysanoessa gregaria	X		X					
Nematoscelis difficilis			X	X		X	X	
Stylocheiron longicorne								
Stylocheiron maximum								

Identification of species was accomplished using a key in Mauchline and Fisher (1969). The entire net haul (100 meter vertical haul) was examined only if very few (10-20) specimens were present. An aliquot part of the haul was examined if the number of euphausiids present was estimated at more than 20 as follows: one half if estimated from 20 to 40; one third if estimated from 40 to 100; and one tenth if estimated more than 100 euphausiids present.

APPENDIX C

SAMPLE XBT TAKEN AT CalCOFI Station 3



SHIP ACANIA

CRUISE 74-14

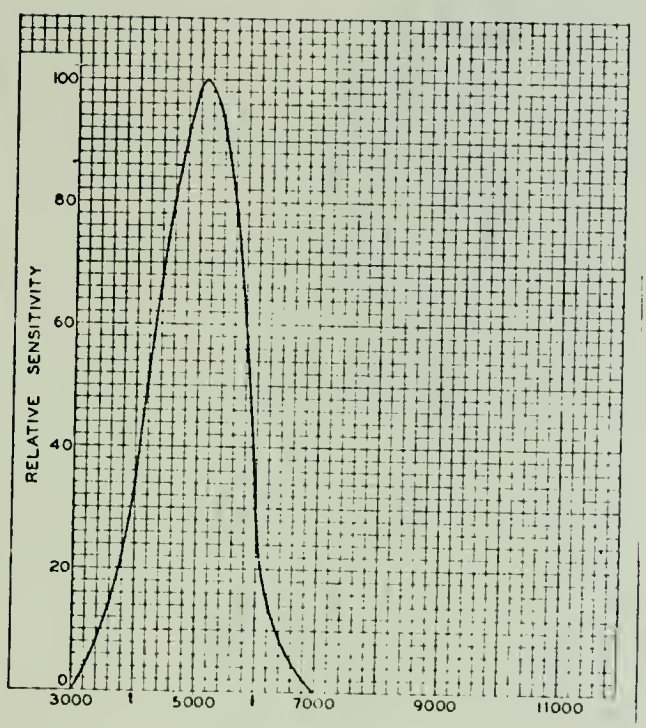
LAT CalCOFI 3

LONG

TIME 1840L

DA/MO/YR 7/2/74

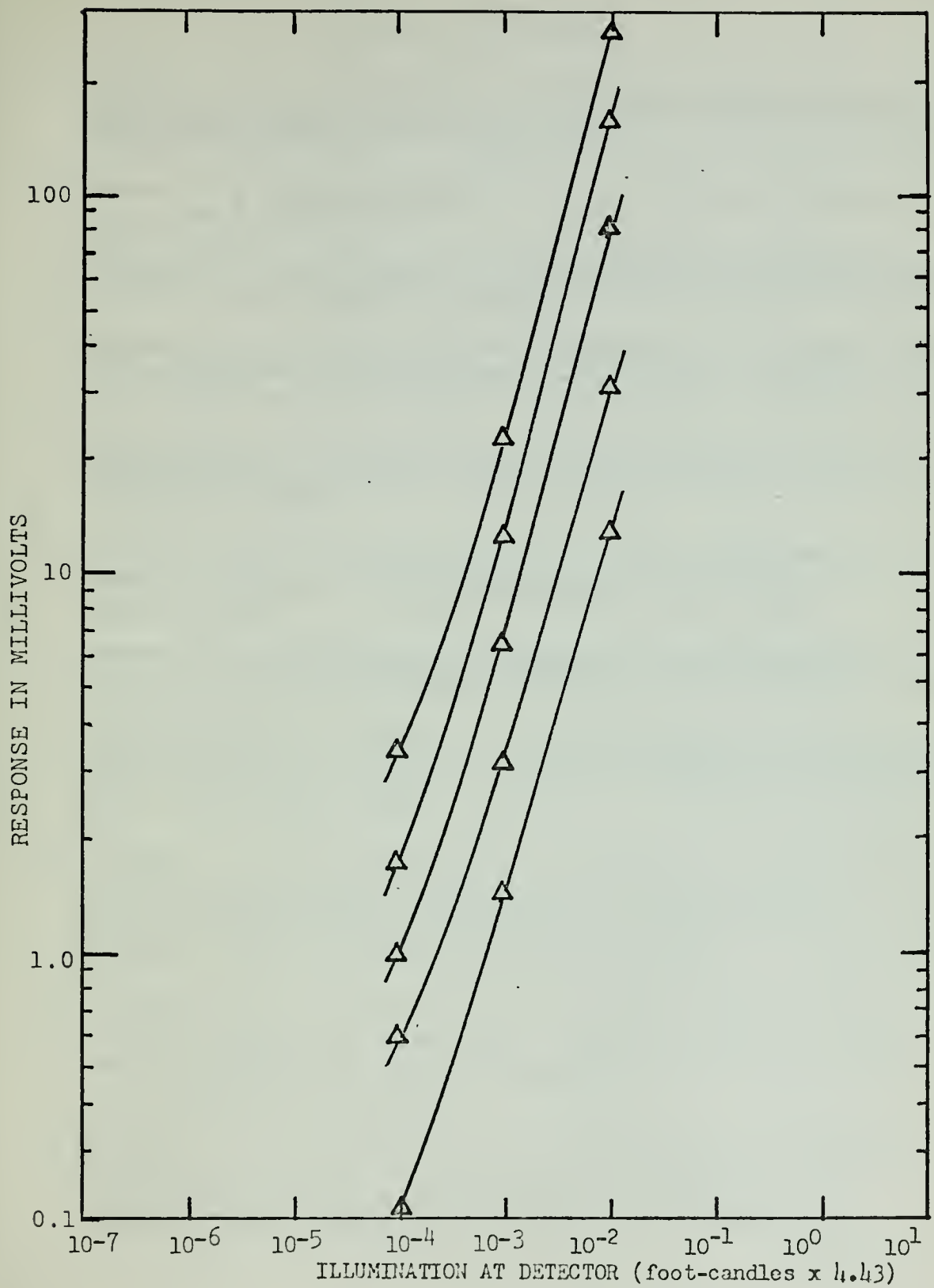
APPENDIX D



Spectral-sensitivity characteristic of phototube having S-4 response to radiant flux from a tungsten source at 2870° K (from RCA data sheet number 92CM-6652R3).

APPENDIX E

Calibration curves for 1P21 photomultiplier tube



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4. TITLE (and Subtitle) A Study of the Bioluminescence of a Deep Scattering Layer Organism (Euphausia pacifica) in Monterey Bay, California		5. TYPE OF REPORT & PERIOD COVERED Master's Thesis; March, 1974 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Andrew Jerome Comptom		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Naval Postgraduate School Monterey, California 93940		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS Naval Postgraduate School Monterey, California 93940		12. REPORT DATE March 1974 13. NUMBER OF PAGES 132
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office) Naval Postgraduate School Monterey, California 93940		15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

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Bioluminescence
Euphausia pacifica
Deep Scattering Layer

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A vertical migration tube (VMT) was designed and constructed as an instrument to be used with a photomultiplier light detector to make in situ mesopelagic studies of deep scattering layer vertical migration organisms. Initial tests of the unit demonstrated the feasibility of its use in the marine environment. Three major problems of marine bioluminescent studies using an underwater photomultiplier detector are resolved in part by the

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(20. ABSTRACT continued)

use of the VMT with this sensor. Mesopelagic euphausiid crustaceans captured in the upper 100 meters of the water column at night decreased their bioluminescent flash rates when lowered in the water column and exposed primarily to pressure and temperature changes. There may be an increase in euphausiid bioluminescent flash rates when stimulated by other bioluminescent organisms. Laboratory test equipment and laboratory methods were developed to permit quantitative measurements of euphausiid bioluminescent output. Laboratory tests of Euphausia pacifica indicated a greater bioluminescent response to a standard flash stimulus during midnight tests as opposed to noon tests. Laboratory tests of bioluminescent activity during periods of moulting indicated greater than average response to a photoflash stimulus just prior to moulting and less than average response just after moulting.

Thesis
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Compton

A study of the bioluminescence of a deep scattering layer organism (Euphausia pacifica) in Monterey Bay, California.